(19) World Intellectual Property Organization International Bureau



(43) International Publication Date 21 March 2002 (21.03.2002)

PCT

(10) International Publication Number WO 02/22080 A2

(51) International Patent Classification7:

A61K

(21) International Application Number: PCT/US01/28861

(22) International Filing Date:

14 September 2001 (14.09.2001)

(25) Filing Language:

English

(26) Publication Language:

Linglish

(30) Priority Data:

60/233,180

15 September 2000 (15.09.2000) US

(71) Applicant (for all designated States except US): MERCK & CO., INC. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US).

(72) Inventors; and

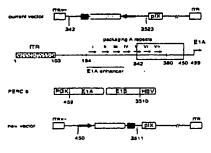
(75) Inventors/Applicants (for US only): EMINI, Emilio, A. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). YOUIL, Rima [AU/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). BETT, Andrew, J.

[CA/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). CHEN, Ling [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). KASLOW, David, C. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). SHIVER, John, W. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). TONER, Timothy, J. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). CASIMIRO, Daniel, R. [PH/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US).

- (74) Common Representative: MERCK & CO., INC.; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US).
- (81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.
- (84) Designated States (regional): ARIPO patent (GII, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian

[Continued on next page]

(54) Title: ENHANCED FIRST GENERATION ADENOVIRUS VACCINES EXPRESSING CODON OPTIMIZED HIV1-GAG, POL, NEF AND MODIFICATIONS



Modifications made to the current adenovactor backbone in the generation of the new

(57) Abstract: First generation adenoviral vectors and associated recombinant adenovirus-based HIV vaccines which show enhanced stability and growth properties and greater cellular-mediated immunity are described within this specification. These adenoviral vectors are utilized to generate and produce through cell culture various adenoviral-based HIV-1 vaccines which contain HIV-1 gag, HIV-1 pol and/or HIV-1 nef polynucleotide pharmaceutical products, and biologically relevant modifications thereof. These adenovirus vaccines, when directly introduced into living vertebrate tissue, preferably a mammalian host such as a human or a non-human mammal of commercial or domestic veterinary importance, express the HIV-1 Gag, Pol and/or Nef protein or biologically modification thereof, inducing a cellular immune response which specifically recognizes HIV-1. The exemplified polynucleotides of the present invention are synthetic DNA molecules encoding HIV-1 Gag, encoding codon optimized HIV-1 Pol, derivatives of optimized HIV-1 Pol (including constructs wherein protease, reverse transcriptase, RNAse H and integrase activity of HIV-1 Pol is inactivated), HIV-1 Nef and derivatives of optimized HIV-1 Nef, including nef mutants which effect wild type characteristics of Nef, such as myristylation and down regulation of host CD4. The adenoviral vaccines of the present invention, when administered alone or in a combined modality regime, will offer a prophylactic advantage to previously uninfected individuals and/or provide a therapeutic effect by reducing viral load levels within an infected individual, thus prolonging the asymptomatic phase of HIV-1 infection.



patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CII, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NI., PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

Published:

 without international search report and to be republished upon receipt of that report

TITLE OF THE INVENTION ENHANCED FIRST GENERATION ADENOVIRUS VACCINES EXPRESSING

CODON OPTIMIZED HIV1-GAG, POL, NEF AND MODIFICATIONS

5 CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit, under 35 U.S.C. §119(e), of U.S. provisional applications 60/233,180, 60/279,056, and Attorney Docket 20867PV2 (serial number unassigned), filed September 15, 2000, March 27, 2001, and September 7, 2001, respectively.

10

15

20

25

30

35

STATEMENT REGARDING FEDERALLY-SPONSORED R&D Not Applicable

REFERENCE TO MICROFICHE APPENDIX

Not Applicable

FIELD OF THE INVENTION

The present invention relates to recombinant, replication-deficient first generation adenovirus vaccines found to exhibit enhanced growth properties and greater cellular-mediated immunity as compared to other replication-deficient vectors. The invention also relates to the associated first generation adenoviral vectors described herein, which, through the incorporation of additional 5' adenovirus sequence, enhance large scale production efficiency of the recombinant, replicationdefective adenovirus described herein. Another aspect of the instant invention is the surprising discovery that the intron A portion of the human cytomegalovirus (hCMV) promoter constitutes a region of instability in adenoviral vector constructs. Removal of this region from adenoviral expression constructs results in greatly improved vector stability. Therefore, improved vectors expressing a transgene under the control of an intron A-deleted CMV promoter constitute a further aspect of this invention. These adenoviral vectors are useful for generating recombinant adenovirus vaccines against human immunodeficiency virus (HIV). In particular, the first generation adenovirus vectors disclosed herein are utilized to construct and generate adenovirus-based HIV-1 vaccines which contain HIV-1 Gag, HIV-1 Pol and/or HIV-1 Nef polynucleotide pharmaceutical products, and biologically active modifications thereof. Host administration of the recombinant, replication-deficient adenovirus vaccines described herein results in expression of HIV-1 Gag, HIV-1- Pol and/or Nef protein or

immunologically relevant modifications thereof, inducing a cellular immune response which specifically recognizes HIV-1. The exemplified polynucleotides of the present invention are synthetic DNA molecules encoding codon optimized HIV-1 Gag, HIV-1 Pol, derivatives of optimized HIV-1 Pol (including constructs wherein protease, reverse transcriptase, RNAse H and integrase activity of HIV-1 Pol is inactivated), HIV-1 Nef, and derivatives of optimized HIV-1 Nef, including nef mutants which effect wild type characteristics of Nef, such as myristylation and down regulation of host CD4. The HIV adenovirus vaccines of the present invention, when administered alone or in a combined modality and/or prime/boost regimen, will offer a prophylactic advantage to previously uninfected individuals and/or provide a therapeutic effect by reducing viral load levels within an infected individual, thus prolonging the asymptomatic phase of HIV-1 infection.

BACKGROUND OF THE INVENTION

10

15

20

25

30

Human Immunodeficiency Virus-1 (HIV-1) is the etiological agent of acquired human immune deficiency syndrome (AIDS) and related disorders. HIV-1 is an RNA virus of the Retroviridae family and exhibits the 5'LTR-gag-pol-env-LTR 3'organization of all retroviruses. The integrated form of HIV-1, known as the provirus, is approximately 9.8 Kb in length. Each end of the viral genome contains flanking sequences known as long terminal repeats (LTRs). The HIV genes encode at least nine proteins and are divided into three classes; the major structural proteins (Gag, Pol, and Env), the regulatory proteins (Tat and Rev); and the accessory proteins (Vpu, Vpr, Vif and Nef).

The gag gene encodes a 55-kilodalton (kDa) precursor protein (p55) which is expressed from the unspliced viral mRNA and is proteolytically processed by the HIV protease, a product of the pol gene. The mature p55 protein products are p17 (matrix), p24 (capsid), p9 (nucleocapsid) and p6.

The pol gene encodes proteins necessary for virus replication; a reverse transcriptase, a protease, integrase and RNAse H. These viral proteins are expressed as a Gag-Pol fusion protein, a 160 kDa precursor protein which is generated via a ribosomal frame shifting. The viral encoded protease proteolytically cleaves the Pol polypeptide away from the Gag-Pol fusion and further cleaves the Pol polypeptide to the mature proteins which provide protease (Pro, P10), reverse transcriptase (RT, P50), integrase (IN, p31) and RNAse H (RNAse, p15) activities.

The *nef* gene encodes an early accessory HIV protein (Nef) which has been shown to possess several activities such as down regulating CD4 expression, disturbing T-cell activation and stimulating HIV infectivity.

The *env* gene encodes the viral envelope glycoprotein that is translated as a 160-kilodalton (kDa) precursor (gp160) and then cleaved by a cellular protease to yield the external 120-kDa envelope glycoprotein (gp120) and the transmembrane 41-kDa envelope glycoprotein (gp41). Gp120 and gp41 remain associated and are displayed on the viral particles and the surface of HIV-infected cells.

5

10

15

20

25

30

35

The *tat* gene encodes a long form and a short form of the Tat protein, a RNA binding protein which is a transcriptional transactivator essential for HIV-1 replication.

The rev gene encodes the 13 kDa Rev protein, a RNA binding protein. The Rev protein binds to a region of the viral RNA termed the Rev response element (RRE). The Rev protein promotes transfer of unspliced viral RNA from the nucleus to the cytoplasm. The Rev protein is required for HIV late gene expression and in turn, HIV replication.

Gp120 binds to the CD4/chemokine receptor present on the surface of helper T-lymphocytes, macrophages and other target cells in addition to other co-receptor molecules. X4 (macrophage tropic) virus show tropism for CD4/CXCR4 complexes while a R5 (T-cell line tropic) virus interacts with a CD4/CCR5 receptor complex. After gp120 binds to CD4, gp41 mediates the fusion event responsible for virus entry. The virus fuses with and enters the target cell, followed by reverse transcription of its single stranded RNA genome into the double-stranded DNA via a RNA dependent DNA polymerase. The viral DNA, known as provirus, enters the cell nucleus, where the viral DNA directs the production of new viral RNA within the nucleus, expression of early and late HIV viral proteins, and subsequently the production and cellular release of new virus particles. Recent advances in the ability to detect viral load within the host shows that the primary infection results in an extremely high generation and tissue distribution of the virus, followed by a steady state level of virus (albeit through a continual viral production and turnover during this phase), leading ultimately to another burst of virus load which leads to the onset of clinical AIDS. Productively infected cells have a half life of several days, whereas chronically or latently infected cells have a 3-week half life, followed by non-productively infected cells which have a long half life (over 100 days) but do not significantly contribute to day to day viral loads seen throughout the course of disease.

Destruction of CD4 helper T lymphocytes, which are critical to immune defense, is a major cause of the progressive immune dysfunction that is the hallmark of HIV infection. The loss of CD4 T-cells seriously impairs the body's ability to fight most invaders, but it has a particularly severe impact on the defenses against viruses, fungi, parasites and certain bacteria, including mycobacteria.

5

10

15

20

25

30

35

Effective treatment regimens for HIV-1 infected individuals have become available recently. However, these drugs will not have a significant impact on the disease in many parts of the world and they will have a minimal impact in halting the spread of infection within the human population. As is true of many other infectious diseases, a significant epidemiologic impact on the spread of HIV-1 infection will only occur subsequent to the development and introduction of an effective vaccine. There are a number of factors that have contributed to the lack of successful vaccine development to date. As noted above, it is now apparent that in a chronically infected person there exists constant virus production in spite of the presence of anti-HIV-1 humoral and cellular immune responses and destruction of virally infected cells. As in the case of other infectious diseases, the outcome of disease is the result of a balance between the kinetics and the magnitude of the immune response and the pathogen replicative rate and accessibility to the immune response. Pre-existing immunity may be more successful with an acute infection than an evolving immune response can be with an established infection. A second factor is the considerable genetic variability of the virus. Although anti-HIV-1 antibodies exist that can neutralize HIV-1 infectivity in cell culture, these antibodies are generally virus isolate-specific in their activity. It has proven impossible to define serological groupings of HTV-1 using traditional methods. Rather, the virus seems to define a serological "continuum" so that individual neutralizing antibody responses, at best, are effective against only a handful of viral variants. Given this latter observation, it would be useful to identify immunogens and related delivery technologies that are likely to elicit anti-HIV-1 cellular immune responses. It is known that in order to generate CTL responses antigen must be synthesized within or introduced into cells, subsequently processed into small peptides by the proteasome complex, and translocated into the endoplasmic reticulum/Golgi complex secretory pathway for eventual association with major histocompatibility complex (MHC) class I proteins. CD8⁺ T lymphocytes recognize antigen in association with class I MHC via the T cell receptor (TCR) and the CD8 cell surface protein. Activation of naive CD8+T cells into activated effector or memory cells generally requires both TCR engagement of antigen as described above as well as engagement of costimulatory proteins. Optimal

induction of CTL responses usually requires "help" in the form of cytokines from CD4⁺ T lymphocytes which recognize antigen associated with MHC class II molecules via TCR and CD4 engagement.

5

10

15

20

25

30 .

35

European Patent Applications 0 638 316 (Published February 15, 1995) and 0 586 076 (Published March 9, 1994), (both assigned to American Home Products Corporation) describe replicating adenovirus vectors carrying an HIV gene, including env or gag. Various treatment regimens were used with chimpanzees and dogs, some of which included booster adenovirus or protein plus alum treatments.

Replication-defective adenoviral vectors harboring deletions in the E1 region are known, and recent adenoviral vectors have incorporated the known packaging repeats into these vectors; e.g., see EP 0 707 071, disclosing, *inter alia*, an adenoviral vector deleted of E1 sequences from base pairs 459 to 3328; and U.S. Patent No. 6,033,908, disclosing, *inter alia*, an adenoviral vector deleted of base pairs 459-3510. The packaging efficiency of adenovirus has been taught to depend on the number of incorporated individual A (packaging) repeats; *see*, *e.g.*, Gräble and Hearing, 1990 *J. Virol.* 64(5):2047-2056; Gräble and Hearing, 1992 *J. Virol.* 66(2):723-731.

Larder, et al., (1987, *Nature* 327: 716-717) and Larder, et al., (1989, *Proc. Natl. Acad. Sci.* 86: 4803-4807) disclose site specific mutagenesis of HIV-1 RT and the effect such changes have on *in vitro* activity and infectivity related to interaction with known inhibitors of RT.

Davies, et al. (1991, *Science* 252:, 88-95) disclose the crystal structure of the RNase H domain of HIV-1 Pol.

Schatz, et al. (1989, *FEBS Lett.* 257: 311-314) disclose that mutations Glu478Gln and His539Phe in a complete HIV-1 RT/RNase H DNA fragment results in defective RNase activity without effecting RT activity.

Mizrahi, et al. (1990, *Nucl. Acids. Res.* 18: pp. 5359-5353) disclose additional mutations Asp443Asn and Asp498Asn in the RNase region of the *pol* gene which also results in defective RNase activity. The authors note that the Asp498Asn mutant was difficult to characterize due to instability of this mutant protein.

Leavitt, et al. (1993, *J. Biol. Chem.* 268: 2113-2119) disclose several mutations, including a Asp64Val mutation, which show differing effect on HIV-1 integrase (IN) activity.

Wiskerchen, et al. (1995, *J. Virol*. 69: 376-386) disclose singe and double mutants, including mutation of aspartic acid residues which effect HIV-1 IN and viral replication functions.

It would be of great import in the battle against AIDS to produce a prophylactic- and/or therapeutic-based HIV vaccine which generates a strong cellular immune response against an HIV infection. The present invention addresses and meets these needs by disclosing a class of adenovirus vaccines which, upon host administration, express codon optimized and modified versions of the HIV-1 genes, gag, pol and nef. These recombinant, replication-defective adenovirus vaccines may be administered to a host, such as a human, alone or as part of a combined modality regimen and/or prime-boost vaccination regimen with components of the present invention and/or a distinct viral HIV DNA vaccine, non-viral HIV DNA vaccine, HIV subunit vaccine, an HIV whole killed vaccine and/or a live attenuated HIV vaccine.

SUMMARY OF THE INVENTION

5

10

15

20

25

30

35

The present invention relates to enhanced replication-defective recombinant adenovirus vaccine vectors and associated recombinant, replication-deficient adenovirus vaccines which encode various forms of HIV-1 Gag, HIV-1 Pol, and/or HIV-1 Nef, including immunologically relevant modifications of HIV-1 Gag, HIV-1 Pol and HIV-1 Nef. The adenovirus vaccines of the present invention express HIV antigens and provide for improved cellular-mediated immune responses upon host administration. Potential vaccinees include but are not limited to primates and especially humans and non-human primates, and also include any non-human mammal of commercial or domestic veterinary importance. An effect of the improved recombinant adenovirus-based vaccines of the present invention should be a lower transmission rate to previously uninfected individuals (i.e., prophylactic applications) and/or reduction in the levels of the viral loads within an infected individual (i.e., therapeutic applications), so as to prolong the asymptomatic phase of HIV-1 infection. In particular, the present invention relates to adenoviral-based vaccines which encode various forms of codon optimized HIV-1 Gag (including but in no way limited to p55 versions of codon optimized full length (FL) Gag and tPA-Gag fusion proteins), HIV-1 Pol, HIV-1 Nef, and selected modifications of immunological relevance. The administration, intracellular delivery and expression of these adenovirus vaccines elicit a host CTL and Th response. The preferred replication-defective recombinant adenoviral vaccine vectors include but are not limited to synthetic DNA molecules which (1) encode codon optimized versions of wild type HIV-1 Gag; (2) encode codon optimized versions of HIV-1 Pol; (3) encode codon optimized versions of HIV-1 Pol fusion proteins; (4) encode codon optimized versions of modified HIV-1 Pol proteins and fusion proteins, including but not limited

to pol modifications involving residues within the catalytic regions responsible for RT, RNase and IN activity within the host cell; (5) encode codon optimized versions of wild type HIV-1 Nef; (6) codon optimized versions of HIV-1 Nef fusion proteins; and/or (7) codon optimized versions of HIV-1 Nef derivatives, including but not limited to nef modifications involving introduction of an amino-terminal leader sequence, removal of an amino-terminal myristylation site and/or introduction of dileucine motif mutations. The Nef-based fusion and modified proteins, disclosed within this specification and expressed from an adenoviral-based vector vaccine this specification, may possess altered trafficking and/or host cell function while retaining the ability to be properly presented to the host MHC I complex and in turn elicit a host CTL and Th response. Examples of HIV-1 Gag, Pol and/or Nef fusion proteins include but are not limited to fusion of a leader or signal peptide at the NH₂-teriminal portion of the viral antigen coding region. Such a leader peptide includes but is not limited to a tPA leader peptide.

10

15

20

25

30

35

The adenoviral vector utilized in construction of the HIV-1 Gag-, HIV-1 Poland/or HIV-1 Nef- based vaccines of the present invention may comprise any replication-defective adenoviral vector which provides for enhanced genetic stability of the recombinant adenoviral genome through large scale production and purification of the recombinant virus. In other words, an HIV-1 Gag-, Pol- or Nef-based adenovirus vaccine of the present invention is a purified recombinant, replicationdefective adenovirus which is shown to be genetically stable through multiple passages in cell culture and remains so during large scale production and purification procedures. Such a recombinant adenovirus vector and harvested adenovirus vaccine lends itself to large scale dose filling and subsequent worldwide distribution procedures which will be demanded of an efficacious monovalent or multivalent HIV vaccine. The present invention meets this basic requirement with description of a replication-defective adenoviral vector and vectors derived therefrom, at least partially deleted in E1, comprising a wildtype adenovirus cis-acting packaging region from about base pair 1 to between from about base pair 342 (more preferably, 400) to about base pair 458 of the wildtype adenovirus genome. A preferred embodiment of the instant invention comprises base pairs 1-450 of a wildtype adenovirus. In other preferred embodiments, the replication -defective adenoviral vector has, in addition thereto, a region 3' to the E1-deleted region comprising base pairs 3511-3523. Basepairs 342-450 (more particularly, 400-450) constitute an extension of the 5'region of previously disclosed vectors carrying viral antigens, particularly HIV antigens (see, e.g., PCT International Application PCT/US00/18332, published

January 11, 2001 (WO 01/02067), which claims priority to U.S. Provisional Application Serial Nos. 60/142,631 and 60/148,981, filed 7/6/1999 and 8/13/1999, respectively; these documents herein incorporated by reference. Applicants have found that extending the 5' region further into the E1 gene into the disclosed vaccine vectors incorporated elements found to be important in optimizing the packaging of the virus.

As compared to previous vectors not comprising basepairs from about 1 to between from about base pair 342 (more preferably, 400) to about base pair 458 of the wildtype adenovirus genome, vectors comprising the above region exhibited enhanced 10 growth characteristics, with approximately 5-10 fold greater amplification rates, a more potent virus effect, allowing lower doses of virus to be used to generate equivalent immunity; and a greater cellular-mediated immune response than replication-deficient vectors not comprising this region (basepairs 1-450). Even more important, adenoviral constructs derived therefrom are very stable genetically in large-scale production, particularly those comprising an expression cassette under the 15 control of a hCMV promoter devoid of intron A. This is because Applicants have surprisingly found that the intron A portion of the hCMV promoter constituted a region of instability when employed in adenoviral vectors. Applicants have, therefore, identified an enhanced adenoviral vector which is particularly suited for use 20 in gene therapy and nucleotide-based vaccine-vectors which, favorably, lends itself to large scale propagation.

A preferred embodiment of this invention is a replication-defective adenoviral vector in accordance with the above description wherein the gene is inserted in the form of a gene expression cassette comprising (a) a nucleic acid encoding a protein or biologically active and/or immunologically relevant portion thereof; (b) a heterologous promoter operatively linked to the nucleic acid of part a); and, (c) a transcription terminator.

25

30

35

In preferred embodiments, the E1 gene, other than that contained within basepairs 1-450 or, alternatively, that contained within base pairs 1-450 and 3511-3523 has been deleted from the adenoviral vector, and the gene expression cassette has replaced the deleted E1 gene. In other preferred embodiments, the replication defective adenovirus genome does not have a functional E3 gene, or the E3 gene has been deleted. Most preferably, the E3 region is present within the adenoviral genome. Further preferred embodiments are wherein the gene expression cassette is in an E1 anti-parallel (transcribed in a 3' to 5' direction relative to the vector backbone)

orientation or, more preferably, an E1 parallel (transcribed in a 5' to 3' direction relative to the vector backbone) orientation.

5

10

15

20

25

30

35

Further embodiments relate to a shuttle plasmid vector comprising: an adenoviral portion and a plasmid portion, wherein said adenovirus portion comprises: a) a replication defective adenovirus genome, at least partially deleted in E1, comprising a wildtype adenovirus cis-acting packaging region from about base pair 1 to between from about base pair 342 (more preferably, 400) to about base pair 458 (preferably, 1-450) of the wildtype adenovirus genome and, preferably, in addition thereto, basepairs 3511-3523 of a wildtype adenovirus sequence; and b) a gene expression cassette comprising: (a) a nucleic acid encoding a protein or biologically active and/or immunologically relevant portion thereof; (b) a heterologous promoter operatively linked to the nucleic acid of part a); and (c) a transcription terminator and/or a polyadenylation site.

Other aspects of this invention include a host cell comprising said adenoviral vectors and/or said shuttle plasmid vectors; vaccine compositions comprising said vectors; and methods of producing the vectors comprising (a) introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and (b) harvesting the resultant adenoviral vectors.

To this end, the present invention particularly relates to harvested recombinant, replication defective virus derived from a host cell, such as but not limited to 293 cells or PER.C6® cells, including but not limited to harvested virus related to any of the MRKAd5 vector backbones, with or without an accompanying transgene, including but not limited to the HIV-1 antigens described herein. An HIV-1 vaccine is represented by any harvested, recombinant adenovirus material which expresses any one or more of the HIV-1 antigens disclosed herein. This harvested material may then be purified, formulated and stored prior to host administration.

Another aspect of this invention is a method of generating a cellular immune response against a protein in an individual comprising administering to the individual an adenovirus vaccine vector comprising:

a) a recombinant, replication defective adenoviral vector, at least partially deleted in E1, comprising a wildtype adenovirus *cis*-acting adenovirus packaging region from about base pair 1 to between from about base pair 342 (more preferably, 400) to about base pair 458 (preferably, 1-450) and, preferably in addition thereto, base pairs 3511-3523 of a wildtype adenovirus sequence, and,

b) a gene expression cassette comprising:(i) a nucleic acid encoding a protein or biologically active and/or immunologically relevant portion thereof; (ii) a heterologous promoter operatively linked to the nucleic acid of part a); and (iii) a transcription terminator and/or a polyadenylation site.

5

10

15

20

25

30

35

In view of the efficacious nature of the adenoviral and/or DNA plasmid vaccines described herein, the present invention relates to all methodology regarding administration of one or more of these adenoviral and/or DNA plasmid vaccines to provide effective immunoprophylaxis, to prevent establishment of an HIV-1 infection following exposure to this virus, or as a post-HIV infection therapeutic vaccine to mitigate the acute HIV-1 infection so as to result in the establishment of a lower virus load with beneficial long term consequences. As discussed herein, such a treatment regimen may include a monovalent or multivalent composition, various combined modality applications, and/or a prime/boost regimen to as to optimize antigen expression and a concomitant cellular-mediated and/or humoral immune response upon inoculation into a living vertebrate tissue. Therefore, the present invention provides for methods of using the adenoviral and/or DNA plasmid vaccines disclosed herein within the various parameters disclosed herein as well as any additional parameters known in the art, which, upon introduction into mammalian tissue induces intracellular expression of the gag, pol and/or nef-based vaccines.

To this end, the present invention relates in part to methods of generating a cellular immune response in a vaccinee, preferably a human vaccinee, wherein the individual is given more than one administration of adenovirus vaccine vector, and it may be given in a regimen accompanied by the administration of a plasmid vaccine. The plasmid vaccine (also referred to herein as a "DNA plasmid vaccine" or "vaccine plasmid" comprises a nucleic acid encoding a protein or an immunologically relevant portion thereof, a heterologous promoter operably linked to the nucleic acid sequence, and a transcription terminator or a polyadenylation signal (such as bGH or SPA, respectively). There may be a predetermined minimum amount of time separating the administrations. The individual can be given a first dose of plasmid vaccine, and then a second dose of plasmid vaccine. Alternatively, the individual may be given a first dose of adenovirus vaccine, and then a second dose of adenovirus vaccine. In other embodiments, the plasmid vaccine is administered first, followed after a time by administration of the adenovirus vaccine. Conversely, the adenovirus vaccine may be administered first, followed by administration of plasmid vaccine after a time. In these embodiments, an individual may be given multiple doses of the same adenovirus serotype in either viral vector or plasmid form, or the virus may be of

differing serotypes. In the alternative, a viral antigen of interest can be first delivered via a viral vaccine other than an adenovirus-based vaccine, and then followed with the adenoviral vaccine disclosed. Alternative viral vaccines include but are not limited to pox virus and venezuelan equine encephilitis virus.

5

10

15

20

25

30

35

The present invention also relates to multivalent adenovirus vaccine compositions which comprise Gag, Pol and Nef components described herein; see, e.g., Example 29 and Table 25. Such compositions will provide for an enhanced cellular immune response subsequent to host administration, particularly given the genetic diversity of human MHCs and of circulating virus. Examples, but not limitations, include MRKAd5-vector based multivalent vaccine compositions which provide for a divalent (i.e., gag and nef, gag and pol, or pol and nef components) or a trivalent vaccine (i.e., gag, pol and nef components) composition. Such a mutlivalent vaccine may be filled for a single dose or may consist of multiple inoculations of each individually filled component; and may in addition be part of a prime/boost regimen with viral or non-viral vector vaccines as introduced in the previous paragraph. To this end, preferred compositions are MRKAd5 adenovirus used in combination with multiple, distinct HIV antigen classes. Each HIV antigen class is subject to sequence manipulation, thus providing for a multitude of potential vaccine combinations; and such combinations are within the scope of the present invention. The utilization of such combined modalities vaccine formulation and administration increase the probability of eliciting an even more potent cellular immune response when compared to inoculation with a single modality regimen.

The concept of a "combined modality" as disclosed herein also covers the alternative mode of administration whereby multiple HIV-1 viral antigens may be ligated into a proper shuttle plasmid for generation of a pre-adenoviral plasmid comprising multiple open reading frames. For example, a trivalent vector may comprise a gag-pol-nef fusion, in either a E3(-) or E3(+) background, preferably a E3 deleted backbone, or possibly a "2+1" divalent vaccine, such as a gag-pol fusion (i.e., codon optimized p55 gag and inactivated optimized pol; Example 29 and Table 25) within the same MRKAd5 backbone, with each open reading frame being operatively linked to a distinct promoter and transcription termination sequence. Alternatively, the two open reading frames may be operatively linked to a single promoter, with the open reading frames operatively linked by an internal ribosome entry sequence (IRES). Therefore, a multivalent vaccine delivered as a single, or possibly a second harvested recombinant, replication-deficient adenovirus is contemplated as part of the present invention.

Therefore, the adenoviral vaccines and plasmid DNA vaccines of this invention may be administered alone, or may be part of a prime and boost administration regimen. A mixed modality priming and booster inoculation scheme will result in an enhanced immune response, particularly if pre-existing anti-vector immune responses are present. This one aspect of this invention is a method of priming a subject with the plasmid vaccine by administering the plasmid vaccine at least one time, allowing a predetermined length of time to pass, and then boosting by administering the adenoviral vaccine. Multiple primings typically, 1-4, are usually employed, although more may be used. The length of time between priming and boost may typically vary from about four months to a year, but other time frames may be used. In experiments with rhesus monkeys, the animals were primed four times with plasmid vaccines, then were boosted 4 months later with the adenoviral vaccine. Their cellular immune response was notably higher than that of animals which had only received adenoviral vaccine. The use of a priming regimen may be particularly preferred in situations where a person has a pre-existing anti-adenovirus immune response.

10

15

20

25

30

35

It is an object of the present invention to provide for enhanced replication-defective recombinant adenoviral vaccine vector backbones. These recombinant adenoviral backbones may accept one or more transgenes, which may be passaged through cell culture for growth, amplification and harvest.

It is a further object to provide for enhanced replication-defective recombinant adenoviral vaccine vectors which encode various transgenes.

It is also an object of the present invention to provide for a harvested recombinant, replication-deficient adenovirus which shows enhanced growth and amplification rates while in combination with increased virus stability after continuous passage in cell culture. Such a recombinant adenovirus is particularly suited for use in gene therapy and nucleotide-based vaccine vectors which, favorably, lends itself to large scale propagation.

To this end, it is an object of the present invention to provide for (1) enhanced replication-defective recombinant adenoviral vaccine vectors as described herein which encode various forms of HIV-1 Gag, HIV-1 Pol, and/or HIV-1 Nef, including immunologically relevant modifications of HIV-1 Gag, HIV-1 Pol and HIV-1 Nef, and (2) harvested, purified recombinant replication-deficient adenovirus generated by passage of the adenoviral vectors of (1) through one or multiple passages through cell culture, including but not limited to passage through 293 cells or PER.C6® cells.

It is also an object of the present invention to provide for recombinant adenovirus harvested by one or multiple passages through cell culture. As relating to recombinant adenoviral vaccine vector, this recombinant virus is harvested and formulated for subsequent host administration.

It is also an object of the present invention to provide for replication-defective adenoviral vectors wherein at least one gene is inserted in the form of a gene expression cassette comprising (a) a nucleic acid encoding a protein or biologically active and/or immunologically relevant portion thereof; (b) a heterologous promoter operatively linked to the nucleic acid of part a); and, (c) a transcription terminator.

5

10

15

20

25

30

35

It is also an object of the present invention to provide for a host cell comprising said adenoviral vectors and/or said shuttle plasmid vectors; vaccine compositions comprising said vectors; and methods of producing the vectors comprising (a) introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and (b) harvesting the resultant adenoviral vectors.

It is a further object of the present invention to provide for methods of generating a cellular immune response against a protein in an individual comprising administering to the individual an adenovirus vaccine vector comprising a) a replication defective

adenoviral vector, at least partially deleted in E1, comprising a wildtype adenovirus cis-acting packaging region from about base pair 1 to between from about base pair 342 (more preferably, 400) to about 450 (preferably, 1-450) and, preferably, 3511-3523 of a wildtype adenovirus sequence, and, b) a gene expression cassette comprising:(i) a nucleic acid encoding a protein or biologically active and/or immunologically relevant portion thereof; (ii) a heterologous promoter operatively linked to the nucleic acid of part a); and (iii) a transcription terminator and/or a polyadenylation site.

It is also an object of the present invention to provide various alternatives for vaccine administration regimes, namely administration of one or more adenoviral and/or DNA plasmid vaccines described herein to provide effective immunoprophylaxis for uninfected individuals or a therapeutic treatment for HIV infected patients. Such processes include but are not limited to multivalent HIV-1 vaccine compositions, various combined modality regimes as well as various prime/boost alternatives. These methods of administration, relating to vaccine composition and/or scheduled administration, will increase the probability of eliciting an even more potent cellular immune response when compared to inoculation with a single modality regimen.

As used throughout the specification and claims, the following definitions and abbreviations are used:

"HAART" refers to -- highly active antiretroviral therapy --.

"first generation" vectors are characterized as being replication-defective.

They typically have a deleted or inactivated E1 gene region, and preferably have a deleted or inactivated E3 gene region as well.

"AEX" refers to Anion Exchange chromatography.

"QPA" refers to Quick PCR-based Potency Assay.

"bps" refers to basepairs.

10

15

20

25

30

35

"s" or "str" denotes that the transgene is in the E1 parallel or "straight" orientation.

"PBMCs" refers to peripheral blood monocyte cells.

"FL" refers to full length.

"FLgag" refers to a full-length optimized gag gene, as shown in Figure 2.

"Ad5-Flgag" refers to an adenovirus serotype 5 replication deficient virus which carries an expression cassette which comprises a full length optimized gag gene under the control of a CMV promoter.

"Promoter" means a recognition site on a DNA strand to which an RNA polymerase binds. The promoter forms an initiation complex with RNA polymerase to initiate and drive transcriptional activity. The complex can be modified by activating sequences such as enhancers or inhibiting sequences such as silencers.

"Leader" means a DNA sequence at the 5' end of a structural gene which is transcribed along with the gene. This usually results a protein having an N-terminal peptide extension, often referred to as a pro-sequences.

"Intron" means a section of DNA occurring in the middle of a gene which does not code for an amino acid in the gene product. The precursor RNA of the intron is excised and is therefore not transcribed into mRNA not translated into protein.

"Immunologically relevant" or "biologically active" means (1) with regards to a viral protein, that the protein is capable, upon administration, of eliciting a measurable immune response within an individual sufficient to retard the propagation and/or spread of the virus and/or to reduce the viral load present within the individual; or (2) with regards to a nucleotide sequence, that the sequence is capable of encoding for a protein capable of the above.

"Cassette" refers to a nucleic acid sequence which is to be expressed, along with its transcription and translational control sequences. By changing the cassette, a vector can express a different sequence.

"bGHpA" refers to the bovine growth hormone transcription terminator/polyadenylation sequence.

"tPAgag" refers to a fusion between the leader sequence of the tissue plasminogen activator leader sequence and an optimized HIV gag gene, as exemplified in Figure 30A-B, whether in a DNA or adenovirus-based vaccine vector.

Where utilized, "IA" or "inact" refers to an <u>inactivated</u> version of a gene (e.g. IApol).

"MCS" is "multiple cloning site".

5

10

15

20

25

30

35

In general, adenoviral constructs, gene constructs are named by reference to the genes contained therein. For example:

"Ad5 HIV-1 gag", also referred to as the original HIV-1 gag adenoviral vector, is a vector containing a transgene cassette composed of a hCMV intron A promoter, the full length version of the human codon-optimized HIV-1 gag gene, and the bovine growth hormone polyadenylation signal. The transgene was inserted in the E1 antiparallel orientation in an E1 and E3 deleted adenovector.

"MRK Ad5 HIV-1 gag" also referred to as "MRKAd5gag" or "Ad5gag2" is an adenoviral vector taught herein which is deleted of E1, comprises basepairs 1-450 and 3511-3523, and has a human codon-optimized HIV-1 gene in an E1 parallel orientation under the control of a CMV promoter without intron A. The construct also comprises a bovine growth hormone polyadenylation signal.

"pV1JnsHIVgag", also referred to as "HIVFLgagPR9901", is a plasmid comprising the CMV immediate-early (IE) promoter and intron A, a full-length codon-optimized HIV gag gene, a bovine growth hormone-derived polyadenylation and transcriptional termination sequence, and a minimal pUC backbone.

"pV1JnsCMV(no intron)-FLgag-bGHpA" is a plasmid derived from pV1JnsHIVgag which is deleted of the intron A portion of CMV and which comprises the full length HIV gag gene. This plasmid is also referred to as "pV1JnsHIVgag-bGHpA", pV1Jns-hCMV-FL-gag-bGHpA" and "pV1JnsCMV(no intron) + FLgag + bGHpA".

"pV1JnsCMV(no intron)-FLgag-SPA" is a plasmid of the same composition as pV1JnsCMV(no intron)-FLgag-bGHpA except that the SPA termination sequence replaces that of bGHpA. This plasmid is also referred to as "pV1Jns-HIVgag-SPA" and pV1Jns-hCMV-FLgag-SPA".

"pdelE1sp1A" is a universal shuttle vector with no expression cassette (i.e., no promoter or polyA). The vector comprises wildtype adenovirus serotype 5 (Ad5) sequences from bp 1 to bp 341 and bp 3524 to bp 5798, and has a multiple cloning

site between the Ad5 sequences ending 341 bp and beginning 3524 bp. This plasmid is also referred to as the original Ad 5 shuttle vector.

"MRKpdelE1sp1A" or "MRKpdelE1(Pac/pIX/pack450)" or

10

15

20

25

30

35

"MRKpdelE1(Pac/pIX/pack450)Cla1" is a universal shuttle vector with no expression cassette (i.e. no promoter or polyA) comprising wildtype adenovirus serotype 5 (Ad5) sequences from bp1 to bp450 and bp 3511 to bp 5798. The vector has a multiple cloning site between the Ad5 sequence ending 450 bp and beginning 3511 bp. This shuttle vector may be used to insert the CMV promoter and the bGHpA fragments in both the straight ("str". or E1 parallel) orientation or in the opposite (opp. or E1 antiparallel) orientation)

"MRKpdelE1(Pac/pIX/pack450)+CMVmin+BGHpA(str.)" is still another shuttle vector which is the modified vector that contains the CMV promoter (no intronA) and the bGHpA fragments. The expression unit containing the hCMV promoter (no intron A) and the bovine growth hormone polyadenylation signal has been inserted into the shuttle vector such that insertion of the gene of choice at a unique *BgI*II site will ensure the direction of transcription of the transgene will be Ad5 E1 parallel when inserted into the MRKpAd5(E1/E3+)Cla1 pre-plasmid. This shuttle vector, as shown in Figures 22 and 23, was used to insert the respective IApol and G2A,LLAA nef genes directly into.

"MRKpdelE1-CMV(no intron)-FLgag-bGHpA" is a shuttle comprising Ad5 sequences from basepairs 1-450 and 3511-5798, with an expression cassette containing human CMV without intron A, the full-length human codon-optimized HIV gag gene and bovine growth hormone polyadenylation signal. This plasmid is also referred to as "MRKpdelE1 shuttle +hCMV-FL-gag-BGHpA"

"MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA" is an adenoviral vector comprising all Ad5 sequences except those nucleotides encompassing the E1 region (from 451-3510), a human CMV promoter without intron A, a full-length human codon-optimized HIV gag gene, and a bovine growth hormone polyadenylation signal. This vector is also referred to as "MRKpAdHVE3 + hCMV-FL-gag-BGHpA", "MRKpAd5HIV-1gag", "MRKpAd5gag", "pMRKAd5gag" or "pAd5gag2".

"pV1Jns-HIV-pol inact(opt)" or "pV1Jns-HIV IA pol (opt) is the inactivated Pol gene (contained within SEQ ID NO:3) cloned into the BgIII site of V1Jns (Figure 17A-C). As noted herein, various derivatives of HIV-1 pol may be cloned into a plasmid expression vector such as V1Jns or V1Jns-tPA, thus serving directly as DNA vaccine candidates or as a source for subcloning into an appropriate adenoviral vector.

"MRKpdel+hCMVmin+FL-pol+bGHpA(s)" is the "MRKpdelE1(Pac/pIX/pack450)+CMVmin+BGHpA(str.)" shuttle mentioned above which contains the IA pol gene is the proper orientation. This shuttle vector is used in a bacterial recombination with MRKpAd(E1-/E3+)Cla1.

"MRKpAd+hCMVmin+FL-pol+bGHpA(S)E3+", also referred to herein as "pMRKAd5pol", is the pre-adenovirus plasmid which comprises a CMV-pol inact(opt)-pGHpA construct. The construction of this pre-adenovirus plasmid is shown in Figure 22.

"pV1Jns/nef (G2A,LLAA)" or "V1Jns/opt nef (G2A,LLAA)" comprises codon optimized HTV-1 Nef wherein the open reading frame codes for modifications at the amino terminal myristylation site (Gly-2 to Ala-2) and substitution of the Leu-174-Leu-175 dileucine motif to Ala-174-Ala-175 (SEQ ID NO:13; which comprises an initiating methionine residue at nucleotides 12-14 and a "TAA" stop codon from nucleotides 660-662). This fragment is subcloned into the Bgl II site of V1Jns and/orV1Jns-tPA (Figures 16A-B). As noted above for HIV-1 pol, HIV-1 nef constructs may be cloned into a plasmid expression vector such as V1Jns or V1Jns-tPA, thus serving directly as DNA vaccine candidates or as a source for subcloning into an appropriate adenoviral vector.

"MRKpdelE1hCMVminFL-nefBGHpA(s)", also referred to herein as "pMRKAd5nef", is the pre-adenovirus plasmid which comprises a CMV-nef (G2A,LLAA) codon optimized sequence. The construction of this pre-adenovirus plasmid is shown in Figure 23.

BRIEF DESCRIPTION OF THE FIGURES

5

10

15

20

25

30

35

Figure 1 shows the original HIV-1 gag adenovector (Ad5HIV-1gag). This vector is disclosed in PCT International Application No. PCT/US00/18332 (WO 01/02607) filed July 3, 2000, claiming priority to U.S. Provisional Application Serial No. 60/142,631, filed July 6, 1999 and U.S. Application Serial No. 60/148,981, filed August 13, 1999, all three applications which are hereby incorporated by reference.

Figure 2 shows the nucleic acid sequence (SEQ ID NO: 29) of the optimized human HIV-1 gag open reading frame.

Figure 3 shows diagrammatically the new transgene constructs in comparison with the original gag transgene.

Figure 4 shows the modifications made to the original adenovector backbone in the generation of the novel vectors of the instant invention.

Figure 5 shows the virus mixing experiments that were carried out to determine the effects of the addition made to the packaging signal region (Expt. #1) and the E3 gene on viral growth (Expt. #2). The bars denote the region of modifications made to the E1 deletion.

Figure 6 shows an autoradiograph of viral DNA analysis following the viral mixing experiments described in Examples 6 and 7.

5

10

15

20

25

30

35

Figures 7A, 7B and 7C are as follows: Figure 7A shows the hCMV-Flgag-bGHpA adenovectors constructed within the MRKpAdHVE3 and MRKpAdHVO adenovector backbones. Both E1 parallel and E1 antiparallel transgene orientation are represented. Figure 7B shows the hCMV-Flgag-SPA adenovectors constructed within the MRKpAdHVE3 and MRKpAdHVO adenovector backbones. Again, both E1 parallel and E1 antiparallel transgene orientation are represented. Figure 7C shows the mCMV-Flgag-bGHpA adenovectors constructed within the MRKpAdHVE3 and MRKpAdHVO adenovector backbones. Once again, both E1 parallel and E1 antiparallel transgene orientation are represented.

Figure 8A shows the experiment designed to test the effect of transgene orientation.

Figure 8B shows the experiments designed to test the effect of polyadenylation signal.

Figure 9 shows viral DNA from the four adenoviral vectors tested (Example 12) at P5, following *Bst*E11 digestion.

Figure 10 shows viral DNA analysis of passages 11 and 12 of MRKpAdHVE3, MRKAd5HIV-1gag, and MRKAd5HIV-1gagE3-.

Figure 11 shows viral DNA analysis (*Hind*III digestion) of passage 6 MRKpAdHVE3 and MRKAd5HIV-1gag used to initiate the viral competition study. The last two lanes are passage 11 analysis of duplicate passages of the competition study (each virus at MOI of 280 viral particles).

Figure 12 shows viral DNA analysis by *Hind* III digestion on high passage numbers for MRKAd5HIV-1gag in serum-containing media with collections made at specified times. The first lane shows the 1kb DNA size marker. The other lanes represent pre-plasmid control (digested with Pac1 and *Hind*III), MRKAd5HIV-1gag at P16, P19, and P21.

Figure 13 shows serum anti-p24 levels at 3 wks post i.m. immunization of balb/c mice (n=10) with varying doses of several Adgag constructs: (A) MRK Ad5 HIV-1 gag (through passage 5); (B) MRKAd5 hCMV-FLgag-bGHpA (E3-); (C) MRKAd5 hCMV-FLgag-SPA (E3+); (D) MRKAd5 mCMV-FLgag-bGHpA (E3+);

(E) research lot (293 cell-derived) of Ad5HIV-1 gag; and (F) clinical lot (Ad5gagFN0001) of Ad5HIV-1 gag. Reported are the geometric mean titers (GMT) for each cohort along with the standard error bars.

Figure 14 shows a restriction map of the pMRKAd5HIV-1gag vector.

Figures 15A-X illustrates the nucleotide sequence of the pMRKAd5HIV-1gag vector (SEQ ID NO:27.[coding] and SEQ ID NO:28 [non-coding]).

5

10

15

20

35

Figures 16A-B shows a schematic representation of DNA vaccine expression vectors V1Ins (A) and V1Ins-tPA (B), which are utilized for HIV-1 gag, pol and nef constructs in various DNA/viral vector combined modality regimens as disclosed herein.

Figures 17A-C shows the nucleotide (SEQ ID NO:3) and amino acid sequence (SEQ ID NO:4) of IA-Pol. Underlined codons and amino acids denote mutations, as listed in Table 1.

Figure 18 shows codon optimized nucleotide and amino acid sequences through the fusion junction of tPA-pol inact(opt) (contained within SEQ ID NOs: 7 and 8, respectively). The underlined portion represents the NH₂-terminal region of IA-Pol.

Figures 19A-B show a nucleotide sequence comparison between wild type nef(jrfl) and codon optimized nef. The wild type nef gene from the jrfl isolate consists of 648 nucleotides capable of encoding a 216 amino acid polypeptide. WT, wild type sequence (SEQ ID NO:19); opt, codon-optimized sequence (contained within SEQ ID NO:1). The Nef amino acid sequence is shown in one-letter code (SEQ ID NO:2).

Figures 20A-C show nucleotide sequences at junctions between nef coding
sequence and plasmid backbone of nef expression vectors V1Jns/nef (Figure 20A),
V1Jns/nef(G2A,LLAA) (Figure 20B), V1Jns/tpanef (Figure 20C) and
V1Jns/tpanef(LLAA) (Figure 20C, also). 5' and 3' flanking sequences of codon
optimized nef or codon optimized nef mutant genes are indicated by bold/italic letters;
nef and nef mutant coding sequences are indicated by plain letters. Also indicated (as
underlined) are the restriction endonuclease sites involved in construction of
respective nef expression vectors. V1Jns/tpanef and V1Jns/tpanef(LLAA) have
identical sequences at the junctions.

Figure 21 shows a schematic presentation of nef and nef derivatives. Amino acid residues involved in Nef derivatives are presented. Glycine 2 and Leucine 174 and 175 are the sites involved in myristylation and dileucine motif, respectively. For both versions of the tpanef fusion genes, the putative leader peptide cleavage sites are

indicated with "*", and a exogenous serine residue introduced during the construction of the mutants is underlined.

Figure 22 shows diagrammatically the construction of the pre-adenovirus plasmid construct, MRKAd5Pol.

Figure 23 shows diagrammatically the construction of the pre-adenovirus plasmid construct, MRKAd5Nef.

5

10

15

20

25

30

35

Figure 24 shows a comparison of clade B vs. clade C anti-gag T cell responses in clade B HIV-infected subjects.

Figure 25 shows a comparison of clade B vs. clade C anti-nef T cell responses in clade B HIV-infected subjects.

Figures 26A-AO illustrates the nucleotide sequence of the pMRKAd5HIV-1pol adenoviral vector (SEQ ID NO:32 [coding] and SEQ ID NO:33 [non-coding]), comprising the coding region of the inactivated pol gene (SEQ ID NO3).

Figures 27A-AM illustrates the nucleotide sequence of the pMRKAd5HIV-1 nef adenoviral vector (SEQ ID NO:34 [coding] and SEQ ID NO:35 [non-coding]), comprising the coding region of the inactivated pol gene (SEQ ID NO13).

Figure 28 shows the stability of MRKAd5 vectors comprising various promoter fragments (hCMV or mCMV) and terminations signals (bGH or SPA) in E3(+) or E3(-) backbones.

Figures 29A and B shows the anion-exchange HPLC viral particle concentrations of the freeze-thaw recovered cell associated virus at the 24, 36, 48, and 60 hpi time points (Figure 29A) and the timcourse QPA supernatant titers (Figure 29B) for MRKAd5gag, MRKAd5pol and MRKAd5nef.

Figure 30 shows the nucleotide sequence (SEQ ID NO:36) and amino acid sequence (SEQ ID NO:37) comprising the open reading frame of a representative tPA-gag fusion for use in the DNA and/or adenoviral vaccine disclosed herein.

Figure 31 shows the intracellular γIFN staining of PBMCs collected at week 10 (post DNA prime) and week 30 (post Ad boost). The cells were stimulated overnight in the presence or absence of the gag peptide pool. They were subsequently stained using fluorescence-tagged anti-CD3, anti-CD8, anti-CD4, and anti-γIFN monoclonal antibodies. Each plot shows all CD3+ T cells which were segregated in terms of positive staining for surface CD8 and γIFN production. The numbers in the upper right and lower right quadrants of each plot are the percentages of CD3⁺ cells that were CD8⁺γIFN⁺ and CD4⁺γIFN⁺, respectively.

Figure 32 shows a comparison of single-modality adenovirus immunization with DNA + adjuvant prime/adenovirus boost immunization.

Figures 33A-B show the nucleotide sequence (SEQ ID NO: 38) of the open reading frame for the gag-IApol fusion of Example 29.

Figures 34A-B show the protein sequence (SEQ ID NO:39) of the gag-IApol fustion frame.

5

10

15

20

25

DETAILED DESCRIPTION OF THE INVENTION

A novel replication-defective, or "first generation," adenoviral vector suitable for use in gene therapy or nucleotide-based vaccine vectors is described. This vector is at least partially deleted in E1 and comprises a wildtype adenovirus cis-acting packaging region from about base pair 1 to between about base pair 342 (more preferably, 400) to about 458 (preferably, 1-450) and, preferably, 3511-3523 of a wild-type adenovirus sequence. It has been found that a vector of this description possesses enhanced growth characteristics, with approximately 5-10 fold greater amplification rates, and is more potent allowing lower doses of virus to be used to generate equivalent immunity. The vector, furthermore, generates a harvested recombinant adenovirus which shows greater cellular-mediated immune responses than replication-deficient vectors not comprising this region (basepairs 342-450). Adenoviral constructs derived from these vectors are, further, very stable genetically, particularly those comprising a transgene under the control of a hCMV promoter devoid of intron A. Viruses in accordance with this description were passaged continually and analyzed; see Example 12. Each virus analyzed maintained it correct genetic structure. Analysis was also carried out under propagation conditions similar to that performed in large scale production. Again, the vectors were found to possess enhanced genetic stability; see Figure 12. Following 21 passages, the viral DNA showed no evidence of rearrangement, and was highly reproducible from one production lot to the next. The outcome of all relevant tests indicate that the adenoviral vector is extremely well suited for large-scale production of recombinant, replication-deficient adenovirus, as shown herein with the data associated with Figure 28.

30

A preferred adenoviral vector in accordance with this description is a vector comprising basepairs 1-450, which is deleted in E3. This vector can accommodate up to approximately 7,500 base pairs of foreign DNA inserts (or exogenous genetic material). Another preferred vector is one retaining E3 which comprises basepairs 1-450. A preferred vector of this description is an E3+ vector comprising basepairs 1-450 and 3511-3523. This vector, when deleted of the region spanning basepairs 451-3510, can accommodate up to approximately, 4,850 base pairs of foreign DNA inserts

(or exogenous genetic material). The cloning capacities of the above vectors have been determined using 105% of the wildtype Ad5 sequence as the upper genome size limit.

Wildtype adenovirus serotype 5 is used as the basis for the specific basepair numbers provided throughout the specification. The wildtype adenovirus serotype 5 sequence is known and described in the art; see, Chroboczek et al., 1992 J. Virology 186:280, which is hereby incorporated by reference. Accordingly, a particular embodiment of the instant invention is a vector based on the adenovirus serotype 5 sequence. One of skill in the art can readily identify the above regions in other adenovirus serotypes (e.g., serotypes 2, 4, 6, 12, 16, 17, 24, 31, 33, and 42), regions defined by basepairs corresponding to the above basepair positions given for adenovirus serotype 5. Accordingly, the instant invention encompasses all adenoviral vectors partially deleted in E1 comprising basepairs corresponding to 1-450 (particularly, 342-450) and, preferably, 3511-3523 of a wild-type adenovirus serotype 5 (Ad5) nucleic acid sequence. Particularly preferred embodiments of the instant invention are those derived from adenoviruses like Ad5 which are classified in subgroup C (e.g., Ad2).

Vectors in accordance with the instant invention are at least partially deleted in E1. Preferably the E1 region is completely deleted or inactivated. Most preferably, the region deleted of E1 is within basepairs 451-3510. It is to be noted that the extended 5' and 3' regions of the disclosed vectors are believed to effectively reduce the size of the E1 deletion of previous constructs without overlapping any part of the E1A/E1B gene present in the cell line used, i.e., the PER.C6® cell line transfected with base pairs 459-3510. Overlap of adenoviral sequences is avoided because of the possibility of recombination. One of ordinary skill in the art can certainly appreciate that the instant invention can, therefore, be modified if a different cell line transfected with a different segment of adenovirus DNA is utilized. For purposes of exemplification, a 5' region of base pairs 1 to up to 449 is more appropriate if a cell line is transfected with adenoviral sequence from base pairs 450-3510. This holds true as well in the consideration of segments 3' to the E1 deletion.

Preferred embodiments of the instant invention possess an intact E3 region (i.e., an E3 gene capable of encoding a functional E3). Alternate embodiments have a partially deleted E3, an inactivated E3 region, or a sequence completely deleted of E3. Applicants have found, in accordance with the instant invention, that virus comprising the E3 gene were able to amplify more rapidly compared with virus not comprising an E3 gene; see Figure 6 wherein a diagnostic CsCl band corresponding to the E3+ virus

tested (5,665 bp) was present in greater amount compared with the diagnostic band of 3,010 bp corresponding to the E3- virus. These results were obtained following a virus competition study involving mixing equal MOI ratio (1:1) of adenovectors both comprising the E3 gene and not comprising the E3 gene. This increased amplification capacity of the E3+ adenovectors was subsequently confirmed with growth studies; see Table 4A, wherein the E3+ virus exhibit amplification ratios of 470, 420 and 320 as compared with the 115 and 40-50 of the E3- constructs.

5

10

15

20

25

30

35

As stated above, vectors in accordance with the instant invention can accommodate up to approximately 4,850 base pairs of exogenous genetic material for an E3+ vector and approximately 7,500 base pairs for an E3- vector. Preferably, the insert brings the adenoviral vector as close as possible to a wild-type genomic size (e.g., for Ad5, 35,935 basepairs). It is well known that adenovirus amplifies best when they are close to their wild-type genomic size.

The genetic material can be inserted in an E1-parallel or an E1 anti-parallel orientation, as such is illustrated in Figure 7A, 7B, 7C and Figure 8A. Particularly preferred embodiments of the instant invention, have the insert in an E1-parallel orientation. Applicants have found, via competition experiments with plasmids containing transgenes in differing orientation (Figure 8A), that vector constructs with the foreign DNA insert in an E1-parallel orientation amplify better and actually outcompete E1-antiparallel-oriented transgenes. Viral DNA analysis of the mixtures at passage 3 and certainly at passage 6, showed a greater ratio of the virus carrying the transgene in the E1 parallel orientation as compared with the E1 anti-parallel version. By passage 10, the only viral species observed was the adenovector with the transgene in the E1 parallel orientation for both transgenes tested.

Adenoviral vectors in accordance with the instant invention are particularly well suited to effectuate expression of desired proteins, one example of which is an HIV protein, particularly an HIV full length gag protein. Exogenous genetic material encoding a protein of interest can exist in the form of an expression cassette. A gene expression cassette preferably comprises (a) a nucleic acid encoding a protein of interest; (b) a heterologous promoter operatively linked to the nucleic acid encoding the protein; and (c) a transcription terminator.

The transcriptional promoter is preferably recognized by an eukaryotic RNA polymerase. In a preferred embodiment, the promoter is a "strong" or "efficient" promoter. An example of a strong promoter is the immediate early human cytomegalovirus promoter (Chapman et al, 1991 *Nucl. Acids Res*19:3979-3986, which is incorporated by reference), preferably without intronic sequences. Most preferred

for use within the instant adenoviral vector is a human CMV promoter without intronic sequences, like intron A. Applicants have found that intron A, a portion of the human cytomegalovirus promoter (hCMV), constitutes a region of instability for adenoviral vectors. CMV without intron A has been found to effectuate (Examples 1-3) comparable expression capabilities *in vitro* when driving HIV gag expression and, furthermore, behaved equivalently to intron A-containing constructs in Balb/c mice *in vivo* with respect to their antibody and T-cell responses at both dosages of plasmid DNA tested (20 µg and 200 µg). Those skilled in the art will appreciate that any of a number of other known promoters, such as the strong immunoglobulin, or other eukaryotic gene promoters may also be used, including the EF1 alpha promoter, the murine CMV promoter, Rous sarcoma virus (RSV) promoter, SV40 early/late promoters and the beta-actin promoter.

In preferred embodiments, the promoter may also comprise a regulatable sequence such as the Tet operator sequence. This would be extremely useful, for example, in cases where the gene products are effecting a result other than that desired and repression is sought.

10

15

20

25

30

35

The combination of the CMV promoter (devoid of the intron A region) with the BGH terminator is particularly preferred although other promoter/terminator combinations in the context of FG adenovirus may also be used.

Other embodiments incorporate a leader or signal peptide into the transgene. A preferred leader is that from the tissue-specific plasminogen activator protein, tPA. Examples include but are not limited to the various tPA-gag, tPA-pol and tPA-nef adenovirus-based vaccines disclosed throughout this specification.

In view of the improved adenovirus vectors described herein, an essential portion of the present invention are adenoviral-based HIV vaccines comprising said adenovirus backbones which may be administered to a mammalian host, preferably a human host, in either a prophylactic or therapeutic setting. The HIV vaccines of the present invention, whether administered alone or in combination regimens with other viral- or non-viral-based DNA vaccines, should elicit potent and broad cellular immune responses against HIV that will either lessen the likelihood of persistent virus infection and/or lead to the establishment of a clinically significant lowered virus load

subject to HIV infection or in combination with HAART therapy, mitigate the effects of previously established HIV infection (antiviral immunotherapy(ARI)). While any HIV antigen (e.g., gag, pol, nef, gp160, gp41, gp120, tat, rev, etc.) may be utilized in the herein described recombinant adenoviral vectors, preferred embodiments include the codon optimized p55 gag antigen (herein exemplified as MRKAd5gag), pol and nef. Sequences based on different Clades of HIV-1 are suitable for use in the instant invention, most preferred of which are Clade B and Clade C. Particularly preferred embodiments are those sequences (especially, codon-optimized sequences) based on concensus Clade B sequences. Preferred versions of the MRKAd5pol and MRKAd5nef series of adenoviral vaccines will encode modified versions of pol or nef, as discussed herein. Preferred embodiments of the MRKAd5HIV-1 vectors carrying HIV envelope genes and modifications thereof comprise the HIV codon-optimized env sequences of PCT International Applications PCT/US97/02294 and PCT/US97/10517, published August 28, 1997 (WO 97/31115) and December 24, 1997, respectively; both documents of which are hereby incorporated by reference.

10

15

20

25

30

35

A most preferred aspect of the instant invention is the disclosed use of the adenoviral vector described above to effectuate expression of HIV gag. Sequences for many genes of many HIV strains are publicly available in GENBANK and primary, field isolates of HIV are available from the National Institute of Allergy and Infectious Diseases (NIAID) which has contracted with Quality Biological (Gaithersburg, MD) to make these strains available. Strains are also available from the World Health Organization (WHO), Geneva Switzerland. It is preferred that the gag gene be from an HIV-1 strain (CAM-1; Myers et al, eds. "Human Retroviruses and AIDS: 1995, IIA3-IIA19, which is hereby incorporated by reference). This gene closely resembles the consensus amino acid sequence for the clade B (North American/European) sequence. Therefore, it is within the purview of the skilled artisan to choose an appropriate nucleotide sequence which encodes a specific HIV gag antigen, or immunologically relevant portion thereof. As shown in Example 25, a clade B or clade C based p55 gag antigen will potentially be useful on a global scale. As noted herein, the transgene of choice for insertion in to a DNA or MRKAd-based adenoviral vector of the present invention is a codon optimized version of p55 gag. Such a MRKAd5gag adenoviral vector is documented in Example 11 and is at least referred to herein as MRKAd5HIV-1gag. Of course, additional versions are contemplated, including but not limited to modifications such as promoter (e.g., mCMV for hCMV) and/or pA-terminations signal (SPA for bGH) switching, as well as generating MRK Ad5 backbones with or without deletion of the Ad5 E3 gene.

The present invention also relates a series of MRKAd5pol-based adenoviral vaccines which are shown herein to generate cellular immune responses subsequent to administration in mice and non-human primate studies. Several of the MRKAd5pol series are exemplified herein. One such adenoviral vector is referred to as MRKAd5hCMV-inact opt pol(E3+), which comprises the MRKAd5 backbone, the 5 hCMV promoter (no intron A), an inactivated pol transgene, and contains the Ad5 E3 gene in the adenoviral backbone. A second exemplified pre-adenovirus plasmid and concomitant virus is referred to as MRKAd5hCMV-inact opt pol(E3-), which is identical to the former adenoviral vector except that the E3 is deleted. Both constructions contain a codon optimized, inactivated version of HIV-1 Pol, wherein at 10 least the entire coding region is disclosed herein as SEQ ID NO:3 and the expressed protein is shown as SEO ID NO:4 (see also Figure 17A-C and Table 1, which show targeted deletion for inactivated pol. This and other preferred codon optimized versions of HIV Pol as disclosed herein are essentially as described in U.S. Application Serial No. 09/745,221, filed December 21, 2000 and PCT International Application PCT/US00/34724, also filed December 21, 2000, both documents which are hereby incorporated by reference. As disclosed in the above-mentioned documents, the open reading frame for these codon-optimized HIV-1 Pol-based DNA vaccines are represented by codon optimized DNA molecules encoding codon optimized HIV-1 Pol (e.g. SEQ ID NO:2), codon optimized HIV-1 Pol fused to an amino terminal localized leader sequence (e.g. SEQ ID NO:6), and especially preferable, and exemplified by the MRKAd5-Pol construct in e.g., Example 19, biologically inactivated pol ("inact opt Pol"; e.g., SEQ ID NO:4) which is devoid of significant PR, RT, RNase or IN activity associated with wild type Pol. In addition, a construct related to SEQ ID NO:4 is contemplated which contains a leader peptide at the amino terminal region of the IA Pol protein. A specific construct is ligated within an appropriate DNA plasmid vector containing regulatory regions operatively linked to the respective HIV-1 Pol coding region, with or without a nucleotide sequence encoding a functional leader peptide. To this end, various HIV-1 Pol constructs disclosed herein relate to open reading frames for cloning to the enhanced first generation Ad vectors of the present invention (such a series of MRKAd5pol adenoviral vaccine vectors), including but not limited to wild type Pol (comprising the DNA molecule encoding WT opt Pol, as set forth in SEQ ID NO:2), tPA-opt WTPol, (comprising the DNA molecule encoding tPA Pol, as set forth in SEQ ID NO:6), inact opt Pol (comprising the DNA molecule encoding IA Pol, as set forth in SEQ ID NO:4), and tPA-inact opt Pol, (comprising the DNA molecule encoding tPA-inact opt

15

20

25

30

35

Pol, as set forth in SEQ ID NO:8). The pol-based versions of enhanced first generation adenovirus vaccines elicit CTL and Th cellular immune responses upon administration to the host, including primates and especially humans. As noted in the above, an effect of the cellular immune-directed vaccines of the present invention should be a lower transmission rate to previously uninfected individuals and/or reduction in the levels of the viral loads within an infected individual, so as to prolong the asymptomatic phase of HIV-1 infection.

5

The present invention further relates to a series of MRKAd5nef-based adenoviral vaccines which, similar to HIV gag and pol antigens, generate cellular immune responses subsequent to administration in mice and non-human primate 10 studies. The MRKAd5nef series are exemplified herein by utilizing the improved MRK adenoviral backbone in combination with modified versions of HIV nef. These exemplified MRKAd5nef vectors are as follows: (1) MRKAd5hCMVnef(G2A,LLAA) (E3+), which comprises the improved MRKAd5 backbone, a human CMV promoter an intact Ad5 E3 gene and a modified nef gene: (2) MRKAd5mCMV-15 nef(G2A,LLAA) (E3+), which is the same as (1) above but substituting a murine CMV promoter for a human CMV promoter; and (3) MRKAd5mCMV-tpanef(LLAA) (E3+), which is the same as (2) except that the nef transgene is tpanef(LLAA). Codon optimized versions of HIV-1 Nef and HIV-1 Nef modifications are essentially as described in U.S. Application Serial No. 09/738,782, filed December 15, 2000 and 20 PCT International Application PCT/US00/34162, also filed December 15, 2000, both documents which are hereby incorporated by reference. Particular embodiments of codon optimized Nef and Nef modifications relate to a DNA molecule encoding HIV-1 Nef from the HIV-1 jfrl isolate wherein the codons are optimized for expression in a mammalian system such as a human. The DNA molecule which encodes this protein 25 is disclosed herein as SEQ ID NO:9, while the expressed open reading frame is disclosed herein as SEQ ID NO:10. Another embodiment of Nef-based coding regions for use in the adenoviral vectors of the present invention comprise a codon optimized DNA molecule encoding a protein containing the human plasminogen activator (tpa) leader peptide fused with the NH2-terminus of the HIV-1 Nef 30 polypeptide. The DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:11, while the expressed open reading frame is disclosed herein as SEQ ID NO:12. Another modified Nef optimized coding region relates to a DNA molecule encoding optimized HIV-1 Nef wherein the open reading frame codes for modifications at the amino terminal myristylation site (Gly-2 to Ala-2) and 35 substitution of the Leu-174-Leu-175 dileucine motif to Ala-174-Ala-175, herein

described as opt nef (G2A, LLAA). The DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:13, while the expressed open reading frame is disclosed herein as SEQ ID NO:14. MRKAd5nef vectors (1) MRKAd5hCMV-nef(G2A,LLAA) (E3+) and (2) MRKAd5mCMV-nef(G2A,LLAA) (E3+) contain this transgene. An additional embodiment relates to a DNA molecule encoding optimized HIV-1 Nef wherein the amino terminal myristylation site and dileucine motif have been deleted, as well as comprising a tPA leader peptide. This DNA molecule, opt tpanef (LLAA), comprises an open reading frame which encodes a Nef protein containing a tPA leader sequence fused to amino acid residue 6-216 of HIV-1 Nef (jfrl), wherein Leu-174 and Leu-175 are substituted with Ala-174 and Ala-175, herein referred to as opt tpanef (LLAA) is disclosed herein as SEQ ID NO:15, while the expressed open reading frame is disclosed herein as SEQ ID NO:16. The MRKAd5nef vector "MRKAd5mCMV-tpanef(LLAA) (E3+)" contains this transgene.

5

10

15

20

25

30

35

Along with the improved MRKAd5gag adenovirus vaccine vector described herein, generation of a MRKAd5pol and MRKAd5nef adenovirus vector provide for enhanced HTV vaccine capabilities. Namely, the generation of this trio of adenoviral vaccine vectors, all shown to generate effective cellular immune responses subsequent to host administration, provide for the ability to administer these vaccine candidates not only alone, but preferably as part of a divalent (i.e., gag and nef, gag and pol, or pol and nef components) or a trivalent vaccine (i.e., gag, pol and nef components). Therefore, a preferred aspect of the present invention are vaccine formulations and associated methods of administration and concomitant generation of host cellular immune responses associated with formulating three separate series of MRKAd5based adenoviral vector vaccines. Of course, this MRKAd5 vaccine series based on distinct HIV antigens promotes expanded opportunities for formulation of a divalent or trivalent vaccine, or possibly administration of separate formulations of one or more monovalent or divalent formulations within a reasonable window of time. It is also within the scope of the present invention to embark on combined modality regimes which include multiple but distinct components from a specific antigen. An example, but certainly not a limitation, would be separate MRKAd5pol vectors, with one vaccine vector expressing wild type Pol (SEQ ID NO:2) and another MRKAd5pol vector expressing inactivated Pol (SEQ ID NO:6). Another example might be separate MRKAd5nef vectors, with one vaccine vector expressing the tPA/LLAA version of Nef (SEQ ID NO:16) and another MRKAd5nef vector expressing the G2A,LLAA modified version of Nef (SEQ ID NO:14). Therefore, the MRKAd5 adenoviral vectors of the present invention may be used in combination

with multiple, distinct HIV antigen classes. Each HIV antigen class is subject to sequence manipulation, thus providing for a multitude of potential vaccine combinations; and such combinations are within the scope of the present invention. The utilization of such combined modalities vaccine formulation and administration increase the probability of eliciting an even more potent cellular immune response when compared to inoculation with a single modality regimen.

5

10

15

20

25

30

35

The present invention also relates to application of a mono-, dual-, or trimodality administration regime of the MRKAd5gag, pol and nef adenoviral vaccine series in a prime/boost vaccination schedule. This prime/boost schedule may include any reasonable combination of the MRKAd5gag, pol and nef adenoviral vaccine series disclosed herein. In addition, a prime/boost regime may also involve other viral and/or non-viral DNA vaccines. A preferable addition to an adenoviral vaccine vector regime includes but is not limited to plasmid DNA vaccines, especially DNA plasmid vaccines that contain at least one of the codon optimized gag, pol and nef constructions, as disclosed herein.

Therefore, one aspect of this invention is the administration of the adenoviral vector containing the optimized gag gene in a prime/boost regiment in conjunction with a plasmid DNA encoding gag. To distinguish this plasmid from the adenoviralcontaining shuttle plasmids used in the construction of an adenovirus vector, this plasmid will be referred to as a "vaccine plasmid" or "DNA plasmid vaccine". Preferred vaccine plasmids for use in this administration protocol are disclosed in pending U.S. patent application 09/017,981, filed February 3, 1998 and WO98/34640, published August 13, 1998, both of which are hereby incorporated by reference. Briefly, the preferred vaccine plasmid is designated V1Jns-FLgag, which expresses the same codon-optimized gag gene as the adenoviral vectors of this invention (see Figure 2 for the nucleotide sequence of the exemplified optimized codon version of full length p55 gag). The vaccine plasmid backbone, designated V1Jns contains the CMV immediate-early (IE) promoter and intron A, a bovine growth hormone-derived polyadenylation and transcription termination sequence as the gene expression regulatory elements, and a minimal pUC backbone; see Montgomery et al., 1993, DNA Cell Biol. 12:777-783. The pUC sequence permits high levels of plasmid production in E. coli and has a neomycin resistance gene in place of an ampicillin resistance gene to provide selected growth in the presence of kanamycin. Alternatively, a vaccine plasmid which has the CMV promoter deleted of intron A can be used. Those of skill in the art will recognize that alternative vaccine plasmid

vectors may be easily substituted for these specific constructs, and this invention specifically envisions use of such alternative plasmid DNA vaccine vectors.

5

10

15

20

25

30

35

Another aspect of the present invention is a prime/boost regimen which includes a vaccine plasmid which encodes an HIV pol antigen, preferably a codon optimized form of pol and also preferably a vaccine plasmid which comprises a nucleotide sequence which encodes a Pol antigen selected from the group of Pol antigens as shown in SEQ ID NOs: 2, 4, 6 and 8. The variety of potential DNA plasmid vaccines which encode various biologically active forms of HIV-1 Pol, wherein administration, intracellular delivery and expression of the HIV-1 Pol gene of interest elicits a host CTL and Th response. The preferred synthetic DNA molecules of the present invention encode codon optimized wild type Pol (without Pro activity) and various codon optimized inactivated HIV-1 Pol proteins. The HIV-1 pol open reading disclosed herein are especially preferred for pharmaceutical uses, especially for human administration as delivered via a recombinant adenoviral vaccine, especially an enhanced first generation recombinant adenoviral vaccine as described herein. Several embodiments of this portion of the invention are provided in detail below, namely DNA molecules which comprise a HIV-1 pol open reading frame, whether encoding full length pol or a modification or fusion as described herein, wherein the codon usage has been optimized for expression in a mammal, especially a human. Again, these DNA sequences are positioned appropriately within a recombinant adenoviral vector, such as the exemplified recombinant adenoviral vector described herein, so as to promote expression of the respective HIV-1 Pol gene of interest, and subsequent to administration, elicit a host CTL and Th response. Again, these preferred, but in no way limiting, pol genes are as disclosed herein and essentially as described in U.S. Application Serial No. 09/745,221, filed December 21, 2000 and PCT International Application PCT/US00/34724, also filed December 21, 2000, both documents which are hereby incorporated by reference.

A third series of vaccine plasmids which are useful in a combined modality and/or prime/boost regimen are vaccine plasmids which encode an HIV nef antigen or biologically and/or immunologically relevant modification thereof. As noted elsewhere, preferred vaccine plasmids contain a codon optimized form of nef and also preferably comprise a nucleotide sequence which encodes a Nef antigen selected from the group of Nef antigens as shown in SEQ ID NOs: 10, 12, 14 and 16. These preferred nef coding regions are disclosed herein, as well as being described in U.S. Application Serial No. 09/738,782, filed December 15, 2000 and PCT International

Application PCT/US00/34162, also filed December 15, 2000, both documents which are hereby incorporated by reference.

5

10

15

Therefore, the adenoviral vaccines and plasmid DNA vaccines of this invention may be administered alone, or may be part of a prime and boost administration regimen. A mixed modality priming and booster inoculation scheme will result in an enhanced immune response, particularly is pre-existing anti-vector immune responses are present. This one aspect of this invention is a method of priming a subject with the plasmid vaccine by administering the plasmid vaccine at least one time, allowing a predetermined length of time to pass, and then boosting by administering the adenoviral vaccine. Multiple primings typically, 1-4, are usually employed, although more may be used. The length of time between priming and boost may typically vary from about four months to a year, but other time frames may be used. In experiments with rhesus monkeys, the animals were primed four times with plasmid vaccines, then were boosted 4 months later with the adenoviral vaccine. Their cellular immune response was notably higher than that of animals which had only received adenoviral vaccine. The use of a priming regimen may be particularly preferred in situations where a person has a pre-existing anti-adenovirus immune response.

Furthermore and in the alternative, multiple HIV-1 viral antigens, such as the MRKAd5 adenoviral vaccines disclosed herein, may be ligated into a proper shuttle 20 plasmid for generation of a pre-adenoviral plasmid comprising multiple open reading frames. For example a trivalent vector may comprise a gag-pol-nef fusion, in either a E3(-) or E3(+) background, preferably a E3 deleted backbone, or possible a "2+1" divalent vaccine, such as a gag-pol fusion (i.e., codon optimized p55 gag and inactivated optimized pol; Example 29 and Table 25) within the same MRKAd5 25 backbone, with each open reading frame being operatively linked to a distinct promoter and transcription termination sequence. Alternatively, the two open reading frames may be operatively linked to a single promoter, with the open reading frames operatively linked by an internal ribosome entry sequence (IRES), as disclosed in International Publication No. WO 95/24485, which is hereby incorporated by 30 reference. Figure 9 shows that the use of multiple promoters and termination sequences provide for similar growth properties, while Figure 28 shows that these MRKAd5gag-based vectors are also stable at least through passage 21. In the absence of the use of IRES-based technology, it is preferred that a distinct promoter be used to support each respective open reading frame, so as to best preserve vector stability. As 35 examples, and certainly not as limitations, potential multiple transgene vaccines may

include a three transgene vector such as hCMV-gagpol-bGHpA + mCMV-nef-SPA in an E3 deleted backbone or hCMV-gagpol-bGHpA + mCMV-nef-SPA(E3+). Potential "2+1" divalent vaccines of the present invention might be a hCMV-gagbGHpA + mCMV-nef-SPA in an E3+ backbone (vector #1) in combination with hCMV-pol-bGHpA in an E3+ backbone (vector #2), with all transgenes in the E1 parallel orientation. Fusion constructs other than the gag-pol fusion described above are also suitable for use in various divalent vaccine strategies and can be composed of any two HIV antigens fused to one another (e.g., nef-pol and gag-nef). These adenoviral compositions are, as above, preferably delivered along with an adenoviral composition comprising an additional HIV antigen in order to diversify the immune response generated upon administration. Therefore, a multivalent vaccine delivered in a single, or possible second, adenoviral vector is certainly contemplated as part of the present invention. Again, this mode of administration is another example of whereby an efficaceous adenovirus-based HIV-1 vaccine may be administered via a combined modality regime. It is important to note, however, that in terms of deciding on an insert for the disclosed adenoviral vectors, due consideration must be dedicated to the effective packaging limitations of the adenovirus vehicle. Adenovirus has been shown to exhibit an upper cloning capacity limit of approximately 105% of the wildtype Ad5 sequence.

10

15

20

25

30

35

Regardless of the gene chosen for expression, it is preferred that the sequence be "optimized" for expression in a human cellular environment. A "triplet" codon of four possible nucleotide bases can exist in 64 variant forms. That these forms provide the message for only 20 different amino acids (as well as transcription initiation and termination) means that some amino acids can be coded for by more than one codon. Indeed, some amino acids have as many as six "redundant", alternative codons while some others have a single, required codon. For reasons not completely understood, alternative codons are not at all uniformly present in the endogenous DNA of differing types of cells and there appears to exist variable natural hierarchy or "preference" for certain codons in certain types of cells. As one example, the amino acid leucine is specified by any of six DNA codons including CTA, CTC, CTG, CTT, TTA, and TTG (which correspond, respectively, to the mRNA codons, CUA, CUC, CUG, CUU, UUA and UUG). Exhaustive analysis of genome codon frequencies for microorganisms has revealed endogenous DNA of E. coli most commonly contains the CTG leucine-specifying codon, while the DNA of yeasts and slime molds most commonly includes a TTA leucine-specifying codon. In view of this hierarchy, it is generally held that the likelihood of obtaining high levels of expression of a leucine-

rich polypeptide by an *E. coli* host will depend to some extent on the frequency of codon use. For example, a gene rich in TTA codons will in all probability be poorly expressed in *E. coli*, whereas a CTG rich gene will probably highly express the polypeptide. Similarly, when yeast cells are the projected transformation host cells for expression of a leucine-rich polypeptide, a preferred codon for use in an inserted DNA would be TTA.

5

10

15

20

25

30

35

The implications of codon preference phenomena on recombinant DNA techniques are manifest, and the phenomenon may serve to explain many prior failures to achieve high expression levels of exogenous genes in successfully transformed host organisms—a less "preferred" codon may be repeatedly present in the inserted gene and the host cell machinery for expression may not operate as efficiently. This phenomenon suggests that synthetic genes which have been designed to include a projected host cell's preferred codons provide a preferred form of foreign genetic material for practice of recombinant DNA techniques. Thus, one aspect of this invention is an adenovirus vector or adenovirus vector in some combination with a vaccine plasmid where both specifically include a gene which is codon optimized for expression in a human cellular environment. As noted herein, a preferred gene for use in the instant invention is a codon-optimized HIV gene and, particularly, HIV gag, pol or nef.

Adenoviral vectors in accordance with the instant invention can be constructed using known techniques, such as those reviewed in Hitt et al, 1997 "Human Adenovirus Vectors for Gene Transfer into Mammalian Cells" Advances in Pharmacology 40:137-206, which is hereby incorporated by reference.

In constructing the adenoviral vectors of this invention, it is often convenient to insert them into a plasmid or shuttle vector. These techniques are known and described in Hitt et al., *supra*. This invention specifically includes both the adenovirus and the adenovirus when inserted into a shuttle plasmid.

Preferred shuttle vectors contain an adenoviral portion and a plasmid portion. The adenoviral portion is essentially the same as the adenovirus vector discussed supra, containing adenoviral sequences (with non-functional or deleted E1 and E3 regions) and the gene expression cassette, flanked by convenient restriction sites. The plasmid portion of the shuttle vector often contains an antibiotic resistance marker under transcriptional control of a prokaryotic promoter so that expression of the antibiotic does not occur in eukaryotic cells. Ampicillin resistance genes, neomycin resistance genes and other pharmaceutically acceptable antibiotic resistance markers may be used. To aid in the high level production of the polynucleotide by

fermentation in prokaryotic organisms, it is advantageous for the shuttle vector to contain a prokaryotic origin of replication and be of high copy number. A number of commercially available prokaryotic cloning vectors provide these benefits. It is desirable to remove non-essential DNA sequences. It is also desirable that the vectors not be able to replicate in eukaryotic cells. This minimizes the risk of integration of polynucleotide vaccine sequences into the recipients' genome. Tissue-specific promoters or enhancers may be used whenever it is desirable to limit expression of the polynucleotide to a particular tissue type.

In one embodiment of this invention, the pre-plasmids (e.g., pMRKAd5pol, pMRKAd5nef and pMRKAd5gag were generated by homologous recombination using the MRKHVE3 (and MRKHVO for the E3- version) backbones and the appropriate shuttle vector, as shown for pMRKAd5pol in Figure 22 and for pMRKAd5nef in Figure 23. The plasmid in linear form is capable of replication after entering the PER.C6® cells and virus is produced. The infected cells and media were harvested after viral replication was complete.

Viral vectors can be propagated in various E1 complementing cell lines, including the known cell lines 293 and PER.C6[®]. Both these cell lines express the adenoviral E1 gene product. PER.C6[®] is described in WO 97/00326 (published January 3, 1997) and issued U.S. Patent No. 6,033,908, both of which are hereby incorporated by reference. It is a primary human retinoblast cell line transduced with an E1 gene segment that complements the production of replication deficient (FG) adenovirus, but is designed to prevent generation of replication competent adenovirus by homologous recombination. Cells of particular interest have been stably transformed with a transgene that encodes the AD5E1A and E1B gene, like PER.C6[®], from 459 bp to 3510 bp inclusive. 293 cells are described in Graham et al., 1977 J. Gen. Virol 36:59-72, which is hereby incorporated by reference. As stated above, consideration must be given to the adenoviral sequences present in the complementing cell line used. It is important that the sequences not overlap with that present in the vector if the possibility of recombination is to be minimized.

It has been found that vectors generated in accordance with the above description are more effective in inducing an immune response and, thus, constitute very promising vaccine candidates. More particularly, it has been found that first generation adenoviral vectors in accordance with the above description carrying a codon-optimized HIV gag gene, regulated with a strong heterologous promoter can be used as human anti-HIV vaccines, and are capable of inducing immune responses.

Standard techniques of molecular biology for preparing and purifying DNA constructs enable the preparation of the DNA immunogens of this invention.

5

15

20

25

30

35

A vaccine composition comprising an adenoviral vector in accordance with the instant invention may contain physiologically acceptable components, such as buffer, normal saline or phosphate buffered saline, sucrose, other salts and polysorbate. One preferred formulation has: 2.5-10 mM TRIS buffer, preferably about 5 mM TRIS buffer; 25-100 mM NaCl, preferably about 75 mM NaCl; 2.5-10% sucrose, preferably about 5% sucrose; 0.01 -2 mM MgCl₂; and 0.001%-0.01% polysorbate 80 (plant derived). The pH should range from about 7.0-9.0, preferably about 8.0. One skilled in the art will appreciate that other conventional vaccine excipients may also be used it make the formulation. The preferred formulation contains 5mM TRIS, 75 mM NaCl, 5% sucrose, 1mM MgCl₂, 0.005% polysorbate 80 at pH 8.0 This has a pH and divalent cation composition which is near the optimum for Ad5 stability and minimizes the potential for adsorption of virus to a glass surface. It does not cause tissue irritation upon intramuscular injection. It is preferably frozen until use.

The amount of adenoviral particles in the vaccine composition to be introduced into a vaccine recipient will depend on the strength of the transcriptional and translational promoters used and on the immunogenicity of the expressed gene product. In general, an immunologically or prophylactically effective dose of $1x10^7$ to $1x10^{12}$ particles and preferably about $1x10^{10}$ to $1x10^{11}$ particles is administered directly into muscle tissue. Subcutaneous injection, intradermal introduction, impression through the skin, and other modes of administration such as intraperitoneal, intravenous, or inhalation delivery are also contemplated. It is also contemplated that booster vaccinations are to be provided. Following vaccination with HIV adenoviral vector, boosting with a subsequent HIV adenoviral vector and/or plasmid may be desirable. Parenteral administration, such as intravenous, intramuscular, subcutaneous or other means of administration of interleukin-12 protein, concurrently with or subsequent to parenteral introduction of the vaccine compositions of this invention is also advantageous.

The adenoviral vector and/or vaccine plasmids of this invention polynucleotide may be unassociated with any proteins, adjuvants or other agents which impact on the recipients' immune system. In this case, it is desirable for the vector to be in a physiologically acceptable solution, such as, but not limited to, sterile saline or sterile buffered saline. Alternatively, the vector may be associated with an adjuvant known in the art to boost immune responses (i.e., a "biologically effective"

adjuvant), such as a protein or other carrier. Vaccine plasmids of this invention may, for instance, be delivered in saline (e.g., PBS) with or without an adjuvant. Preferred adjuvants are Alum or CRL1005 Block Copolymer. Agents which assist in the cellular uptake of DNA, such as, but not limited to, calcium ions, may also be used to advantage. These agents are generally referred to herein as transfection facilitating reagents and pharmaceutically acceptable carriers. Techniques for coating microprojectiles coated with polynucleotide are known in the art and are also useful in connection with this invention.

10

15

20

25

30

35

This invention also includes a prime and boost regimen wherein a first adenoviral vector is administered, then a booster dose is given. The booster dose may be repeated at selected time intervals. Alternatively, a preferred inoculation scheme comprises priming with a first adenovirus serotype and then boosting with a second adenovirus serotype. More preferably, the inoculation scheme comprises priming with a first adenovirus serotype and then boosting with a second adenovirus serotype, wherein the first and second adenovirus serotypes are classified within separate subgroups of adenoviruses. The above prime/boost schemes are particularly preferred in those situations where a preexisting immunity is identified to the adenoviral vector of choice. In this type of scheme, the individual or population of individuals is primed with an adenovirus of a serotype other than that to which the preexisting immunity is identified. This enables the first adenovirus to effectuate sufficient expression of the transgene while evading existing immunity to the second adenovirus (the boosting adenovirus) and, further, allows for the subsequent delivery of the transgene via the boosting adenovirus to be more effective. Adenovirus serotype 5 is one example of a virus to which such a scheme might be desirable. In accordance with this invention, therefore, one might decide to prime with a non-group C adenovirus (e.g., Ad12, a group A adenovirus, Ad24, a group D adenovirus, or Ad35, a group B adenovirus) to evade anti-Ad5 immunity and then boost with Ad5, a group C adenovirus. Another preferred embodiment involves administration of a different adenovirus (including non-human adenovirus) vaccine followed by administration of the adenoviral vaccines disclosed. In the alternative, a viral antigen of interest can be first delivered via a viral vaccine other than an adenovirus-based vaccine, and then followed with the adenoviral vaccine disclosed. Alternative viral vaccines include but are not limited to pox virus and venezuelan equine encephilitis virus.

A large body of human and animal data supports the importance of cellular immune responses, especially CTL in controlling (or eliminating) HIV infection. In humans, very high levels of CTL develop following primary infection and correlate

with the control of viremia. Several small groups of individuals have been described who are repeatedly exposed to HIV by remain uninfected; CTL has been noted in several of these cohorts. In the SIV model of HIV infection, CTL similarly develops following primary infection, and it has been demonstrated that addition of anti-CD8 monoclonal antibody abrogated this control of infection and leads to disease progression. This invention uses adenoviral vaccines alone or in combination with plasmid vaccines to induce CTL.

The following non-limiting Examples are presented to better illustrate the invention.

10

15

20

25

30

35

EXAMPLE 1 Removal of the Intron A Portion of the hCMV Promoter

GMP grade pVIInsHIV gag was used as the starting material to amplify the

hCMV promoter. PVIInsHIVgag is a plasmid comprising the CMV immediate-early (IE) promoter and intron A, a full-length codon-optimized HIV gag gene, a bovine growth hormone-derived polyadenylation and transcriptional termination sequence, and a minimal pUC backbone; see Montgomery et al., supra for a description of the plasmid backbone. The amplification was performed with primers suitably positioned to flank the hCMV promoter. A 5' primer was placed upstream of the Msc1 site of the hCMV promoter and a 3' primer (designed to contain the Bg/II recognition sequence) was placed 3' of the hCMV promoter. The resulting PCR product (using high fidelity Taq polymerase) which encompassed the entire hCMV promoter (minus

intron A) was cloned into TOPO PCR blunt vector and then removed by double digestion with Msc1 and BgIII. This fragment was then cloned back into the original GMP grade pV1JnsHIVgag plasmid from which the original promoter, intron A, and the gag gene were removed following Msc1 and BgIII digestion. This ligation reaction resulted in the construction of a hCMV promoter (minus intron A) + bGHpA expression cassette within the original pV1JnsHIVgag vector backbone. This vector

is designated pVIInsCMV(no intron).

The FLgag gene was excised from pV1JnsHIVgag using BgIII digestion and the 1,526 bp gene was gel purified and cloned into pV1JnsCMV(no intron) at the BgIII site. Colonies were screened using Sma1 restriction enzymes to identify clones that carried the Flgag gene in the correct orientation. This plasmid, designated pV1JnsCMV(no intron)-FLgag-bGHpA, was fully sequenced to confirm sequence integrity.

Two additional transgenes were also constructed. The plasmid, pV1JnsCMV(no intron)-FLgag-SPA, is identical to pV1JnsCMV(no intron)-FLgag-bGHpA except that the bovine growth hormone polyadenylation signal has been replaced with a short synthetic polyA signal (SPA) of 50 nucleotides in length. The sequence of the SPA is as shown, with the essential components (poly(A) site, (GT)_n, and (T)_n; respectively) underlined:

<u>AATAAA</u>AGATCTTTATTTTCATTAGATCT<u>GTGTG TTGGTTTTTTGTGTG</u> (SEO ID NO:18).

The plasmid, pV1Jns-mCMV-FLgag-bGHpA, is identical to the pV1JnsCMV(no intron)-FLgag-bGHpA except that the hCMV promoter has been removed and replaced with the murine CMV (mCMV) promoter.

Figure 3 diagrammatically shows the new transgene constructs in comparison with the original transgene.

15 EXAMPLE 2

5

10

20

25

Gag Expression Assay for Modified Gag Transgenes

Gag Elisa was performed on culture supernatants obtained from transient tissue culture transfection experiments in which the two new hCMV-containing plasmid constructs, pV1JnsCMV(no intron)-FLgag-bGHpA and pV1JnsCMV(no intron)-FLgag-SPA, both devoid of intron A, were compared to pV1JnsHIVgag which, as noted above possesses the intron A as part of the hCMV promoter. Table 2 below shows the *in vitro* gag expression data of the new gag plasmids compared with the GMP grade original plasmid. The results displayed in Table 2 show that both of the new hCMV gag plasmid constructs have expression capacities comparable to the original plasmid construct which contains the intron A portion of the hCMV promoter.

Table 2: In vitro DNA transfection of original and new plasmid HIV-1 gag constructs.

Plasmid	μg gag/10e6 COS cells/5μg DNA/48 hr
HIVFL-gagPR9901 ^a	10.8
PVIIns-hCMV-FLgag-bGHpAb	16.6
pV1Jns-hCMV-FLgag-SPA ^{b,c}	12.0

^a GMP grade pV1Jns-hCMVintronA-FLgag-bGHpA.

10

15

20

EXAMPLE 3

Rodent (Balb/c) Study for Modified gag Transgenes
A rodent study was performed on the two new plasmid constructs
described above – pV1JnsCMV(no intron)-FLgag-bGHpA and pV1JnsCMV(no
intron)-FLgag-SPA - in order to compare them with the construct described above
possessing the intron A portion of the CMV promoter, pV1JnsHIVgag. Gag antibody
and Elispot responses (described in PCT International Application No.
PCT/US00/18332 (WO 01/02607) filed July 3, 2000, claiming priority to U.S.
Provisional Application Serial No. 60/142,631, filed July 6, 1999 and U.S.
Application Serial No. 60/148,981, filed August 13, 1999, all three applications which
are hereby incorporated by reference) were measured. The results displayed in Table
3 below, show that the new plasmid constructs behaved equivalently to the original
construct in Balb/c mice with respect to their antibody and T-cell responses at both
dosages of plasmid DNA tested, 20 μg and 200 μg.

⁵ New plasmid constructions that have the intron A portion removed from the hCMV promoter.

^c In this construct the bGH terminator has been replaced with the short synthetic polyadenylation signal (SPA)

EXAMPLE 4

Table 3: HIV191: Immunogenicity of V1Jns-gag under different promoter and termination control elements.

DNA®	Dose,	Anti-p24 Titers			SFC/10^6 Cells			
	ug ^b	(3 Wk PD1)°			(4 Wk PD1) ^d			
Promoter/terminator		GMT	+SE	-\$E	Media	gag197-205	p24	
HIVFL-gagPR9901	200	12800	4652	3412	2(2)	129(19)	30(11)	
(GMP grade)	20	5572	1574	1227	0	56(9)	25(6)	
pV1Jns-hCMV-	200	11143	2831	2257	0	98(5)	12(6)	
FL-gag-bGHpA	20	7352	2808	2032	0	73(9)	11(6)	
pV1Jns-hCMV-	200	16890	5815	4326	1(1)	94(4)	26(7)	
FL-gag-SPA	20	5971	5361	2825	0	85(17)	38(10)	
Naīve	0	123	50	36	0	0	0	

in PBS

5

20

Construction of the Modified Shuttle Vector - "MRKpdelE1 Shuttle"

The modifications to the original Ad5 shuttle vector (pdelE1sp1A; a vector comprising Ad5 sequences from basepairs 1-341 and 3524-5798, with a multiple cloning region between nucleotides 341 and 3524 of Ad5, included the following three manipulations carried out in sequential cloning steps as follows:

- (1) The left ITR region was extended to include the *Pac1* site at the junction between the vector backbone and the adenovirus left ITR sequences. This allow for easier manipulations using the bacterial homologous recombination system.
- 10 (2) The packaging region was extended to include sequences of the wild-type (WT) adenovirus from 342 bp to 450 bp inclusive.
 - (3) The area downstream of pIX was extended 13 nucleotides (i.e., nucleotides 3511-3523 inclusive).

These modifications (Figure 4) effectively reduced the size of the E1 deletion without overlapping with any part of the E1A/E1B gene present in the transformed PER.C6[®] cell line. All manipulations were performed by modifying the Ad shuttle vector pdelE1sp1A.

Once the modifications were made to the shuttle vector, the changes were incorporated into the original Ad5 adenovector backbones (pAdHVO and pAdHVE3) by bacterial homologous recombination using *E. coli* BJ5183 chemically competent cells.

bi.m. Injections into both quads, 50 μL per quad

[°]n=10;GMT, geometric mean titer; SE, standard. error

dn=5, pooled spleens; mean of triplicate wells and standard. deviation. in parentheses;

EXAMPLE 5

Construction of Modified Adenovector Backbones (E3+ and E3-)

The original adenovectors pAdHVO (comprising all Ad5 sequences except those nucleotides encompassing the E1 and E3 regions) and pADHVE3 (comprising all Ad5 sequences except those nucleotides encompassing the E1 region), were each 5 reconstructed so that they contained the modifications to the E1 region. This was accomplished by digesting the newly modified shuttle vector (MRKpdelE1 shuttle) with Pac1 and BstZ1101 and isolating the 2,734 bp fragment which corresponds to the adenovirus sequence. This fragment was co-transformed with DNA from either Cla1 10 linearized pAdHVO (E3- adenovector) or Cla1 linearized pAdHVE3 (E3+adenovector) into E. coli BJ5183 competent cells. At least two colonies from each transformation were selected and grown in Terrific™ broth for 6-8 hours until turbidity was reached. DNA was extracted from each cell pellet and then transformed into E. coli XL1 competent cells. One colony from each transformation was selected and grown for plasmid DNA purification. The plasmid was analyzed by restriction 15 digestions to identify correct clones. The modified adenovectors were designated MRKpAdHVO (E3- plasmid) and MRKpAdHVE3 (E3+ plasmid). Virus from these new adenovectors (MRKHVO and MRKHVE3, respectively) as well as the old version of the adenovectors were generated in the PER.C6® cell lines to accommodate the following series of viral competition experiments. In addition, the multiple 20 cloning site of the original shuttle vector contained ClaI, BamHI, Xho I, EcoRV, HindIII, Sal I, and Bgl II sites. This MCS was replaced with a new MCS containing Not I, Cla I, EcoRV and Asc I sites. This new MCS has been transferred to the MRKpAdHVO and MRKpAdHVE3 pre-plasmids along with the modification made to the packaging region and pIX gene. 25

EXAMPLE 6

Analysis of the Effect of the Packaging Signal Extension

To study the effects of the modifications made to the E1 deletion region, the viruses obtained from the original backbone (pAdHVE3) and the new backbone (MRKpAdHVE3) were mixed together in equal MOI ratios (1:1 and 5:5) and passaged through several rounds; see Figure 5, Expt.#1. Both of the viruses in the experiment contained the E3 gene intact and did not contain a transgene. The only difference between the two viruses was within the region of the E1 deletion. Following the coinfection of the viruses at P1 (passage 1), the mixtures were

30

35

propagated through an additional 4 passages at which time the cells were harvested

and the virus extracted and purified by CsCl banding. The viral DNA was extracted and digested with *Hind*III and the digestion products were then radioactively labeled. For the controls, the respective pre-plasmids (pAdHVE3 ("OLD E3+"); MRKpAdHVE3 ("NEW E3+")) were also digested with *Hind*III (and *Pac1* to remove the vector backbone) and subsequently labeled with [³³P]dATP. The radioactively labeled digestion products were subjected to gel electrophoresis and the gel was dried down onto Whatman paper before being exposed to autoradiographic film. Figure 6 clearly shows that the new adenovirus which has the addition made to the packaging signal region has a growth advantage compared with the original adenovirus. In the experiments performed (at either ratio tested), only the digestion bands pertaining to the newly modified virus were present. The diagnostic band of size 3,206 (from the new virus) was clearly present. However, there was no evidence of the diagnostic band of size 2,737 bp expected from the original virus.

15

20

25

30

35

10

5

EXAMPLE 7

Analysis of the Effect of the E3 Gene

The second set of the virus competition study involved mixing equal MOI ratio (1:1) of the newly modified viruses, that obtained from MRKpAdHVO and MRKpAdHVE3 (Figure 5, Expt. #2). In this set, both viruses had the new modifications made to the E1 deletion. The first virus (that from MRKpAdHVO) does not contain an E3 gene. The second virus (that from MRKpAdHVE3) does contain the E3 gene. Neither of the viruses contain a transgene. Following coinfection of the viruses, the mixtures were propagated through an additional 4 passages at which time the cells were harvested and the total virus extracted and purified by CsCl banding. The viral DNA was extracted and digested with HindIII and the digestion products were then radioactively labeled. For the controls, the respective pre-plasmids MRKpAdHVO ("NEW E3-"); MRKpAdHVE3 ("NEW E3+") were also digested with HindIII (and Pac1 to remove the vector backbone) and then labeled with [33P]dATP. The radioactively labeled digestion products were subjected to gel electrophoresis and the gel was dried down onto Whatman paper before being exposed to autoradiographic film. Figure 6 shows the results of the viral DNA analysis of the E3+ virus and E3- virus mixing experiment. The diagnostic band corresponding to the E3+ virus (5,665 bp) was present in greater amount compared with the diagnostic band of 3,010 bp corresponding to the E3- virus. This indicates that the virus that contains the E3 gene is able to amplify more rapidly

compared with the virus that does not contain an E3 gene. This increased amplification capacity has been confirmed by growth studies; see Table 4 below.

EXAMPLE 8

5 <u>Construction of the new shuttle vector containing modified gag transgene</u> — "MRKpdelE1-CMV(no intron)-FLgag-bGHpA"

The modified plasmid pV1JnsCMV(no intron)-FLgag-bGHpA was digested with Msc1 overnight and then digested with Sfi1 for 2 hours at 50°C. The DNA was then treated with Mungbean nuclease for 30 mins at 30°C. The DNA mixture was desalted using the Qiaex II kit and then Klenow treated for 30 mins at 37°C to fully blunt the ends of the transgene fragment. The 2,559 bp transgene fragment was then gel purified. The modified shuttle vector (MRKpdelE1 shuttle) was linearized by digestion with EcoRV, treated with calf intestinal phosphatase and the resulting 6,479 bp fragment was then gel purified. The two purified fragments were then ligated together and several dozen clones were screened to check for insertion of the transgene within the shuttle vector. Diagnostic restriction digestion was performed to identify those clones carrying the transgene in the E1 parallel and E1 anti-parallel orientation. This strategy was followed to clone in the other gag transgenes in the MRKpdelE1 shuttle vector.

20

25

30

35

15

10

EXAMPLE 9

Construction of the MRK FG Adenovectors

The shuttle vector containing the HIV-1 gag transgene in the E1 parallel orientation, MRKpdelE1-CMV(no intron)-FLgag-bGHpA, was digested with *Pac1*. The reaction mixture was digested with *BsfZ*171. The 5,291 bp fragment was purified by gel extraction. The MRKpAdHVE3 plasmid was digested with *Cla1* overnight at 37°C and gel purified. About 100 ng of the 5,290 bp shuttle +transgene fragment and ~100 ng of linearized MRKpAdHVE3 DNA were co-transformed into *E. coli* BJ5183 chemically competent cells. Several clones were selected and grown in 2 ml TerrificTM broth for 6-8 hours, until turbidity was reached. The total DNA from the cell pellet was purified using Qiagen alkaline lysis and phenol chloroform method. The DNA was precipitated with isopropanol and resuspended in 20 μl dH₂0. A 2 μl aliquot of this DNA was transformed into *E. coli* XL-1 competent cells. A single colony from each separate transformation was selected and grown overnight in 3 ml LB +100 μg/ml ampicillin. The DNA was isolated using Qiagen columns. A positive clone was identified by digestion with the restriction enzyme *Bst*EII which cleaves

within the gag gene as well as the plasmid backbone. The pre-plasmid clone is designated MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA and is 37,498 bp in size. This strategy was followed to generate E3- and E3+ versions of each of the other gag transgene constructions in both E1 parallel and E1 anti-parallel versions. Figures 7A, 7B and 7C show the various combinations of adenovectors constructed.

EXAMPLE 10

Plasmid Competition Studies

A series of plasmid competition studies was carried out. Briefly, the screening of the various combinations of new constructs was performed by mixing equal amounts of each of two competing plasmids. In the experiment shown in Figure 8A, plasmids containing the same transgene but in different orientations were mixed together to create a "competition" between the two plasmids. The aim was to look at the effects of transgene orientation. In the experiment shown in Figure 8B, plasmids containing different polyadenylation signals (but in the same orientation) were mixed together in equal amounts. The aim was to assess effects of polyA signals. Following the initial transfection, the virus was passaged through ten rounds and the viral DNA analyzed by radioactive restriction analysis.

Analysis of the viral species from the plasmid mixing experiment (Figure 8A) showed that adenovectors which had the transgene inserted in the E1 parallel orientation amplified better and were able to out-compete the adenovirus which had the transgene inserted in the E1 anti-parallel orientation. Viral DNA analysis of the mixtures at passage 3 and certainly at passage 6, showed a greater ratio of the virus carrying the transgene in the E1 parallel orientation compared with the E1 antiparallel version. By passage 10, the only viral species observed was the adenovector with the transgene in the E1 parallel orientation for both transgenes tested (hCMV(no intron)-FLgag-bGHpA and hCMV(no intron)-FLgag-SPA).

Analysis of the viral species from the plasmid mixing experiment #2 (Figure 8B) at passages 3 and 6 showed that the polyadenylation signals tested (bGHpA and SPA) did not have an effect on the growth of the virus. Even at passage 10 the two viral species in the mixture were still present in equal amounts.

30

5

10

15

20

25

EXAMPLE 11

Virus generation of an enhanced adenoviral construct - "MRK Ad5 HIV-1gag"

The results obtained from the competition study allowed us to make the following conclusions: (1) The packaging signal extension is beneficial; (2) Presence of E3 does enhance viral growth; (3) E1 parallel orientation is recommended; and (4) PolyA signals have no effect on the growth of the adenovirus.

MRK Ad5 HIV-1 gag exhibited the most desirable results. This construct contains the hCMV(no intron)-FLgag-bGHpA transgene inserted into the new E3+ adenovector backbone, MRKpAdHVE3, in the E1 parallel orientation. We have designated this adenovector MRK Ad5 HIV-1 gag. This construct was prepared as outlined below:

The pre-plasmid MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA was digested was Pac1 to release the vector backbone and 3.3 µg was transfected by calcium phosphate method (Amersham Pharmacia Biotech.) in a 6 cm dish containing PER.C6[®] cells at ~60% confluence. Once CPE was reached (7-10 days), the culture was freeze/thawed three times and the cell debris pelleted. 1 ml of this cell lysate was used to infect into a 6 cm dish containing PER.C6® cells at 80-90% confluence. Once CPE was reached, the culture was freeze/thawed three times and the cell debris pelleted. The cell lysate was then used to infect a 15 cm dish containing PER.C6[®] cells at 80-90% confluence. This infection procedure was continued and expanded at passage 6. The virus was then extracted from the cell pellet by CsCl method. Two bandings were performed (3-gradient CsCl followed by a continuous CsCl gradient). Following the second banding, the virus was dialyzed in A105 buffer. Viral DNA was extracted using pronase treatment followed by phenol chloroform. The viral DNA was then digested with *Hind*III and radioactively labeled with [33P]dATP. Following gel electrophoresis to separate the digestion products the gel was dried down on Whatman paper and then subjected to autoradiography. The digestion products were compared with the digestion products from the pre-plasmid (that had been digested with Pac1/HindIII prior to labeling). The expected sizes were observed, indicating that the virus had been successfully rescued. This strategy was used to rescue virus from each of the various adenovector plasmid constructs prepared.

5

10

15

20

25

30

EXAMPLE 12

Stability Analyses

5

10

15

20

25

30

35

To determine whether the various adenovector constructs (e.g., MRK Ad5 HIV-1 gag) show genetic stability, the viruses were each passaged continually. The viral DNA was analyzed at passages 3, 6 and 10. Each virus maintained its correct genetic structure. In addition, the stability of the MRK Ad5 HIV-1 gag was analyzed under propagation conditions similar to that performed in large scale production. For this analysis, the transfections of MRK Ad5 HIV-1 gag as well as three other adenoviral vectors were repeated and the virus was purified at P3. The three other adenovectors were as follows: (1) that comprising hCMV(no intron)-Flgag with a bGHpA terminator in an E3- adenovector backbone; (2) that comprising hCMV(no intron)-Flgag with a SPA termination signal in an E3+ adenovector backbone, and that comprising a mCMV-Flgag with a bGHpA terminator in an E3+ adenovector backbone. All of the vectors have the transgene inserted in the E1 parallel orientation. Viral DNA was analyzed by radioactive restriction analysis to confirm that it was correct before being delivered to fermentation cell culture for continued passaging in serum-free media. At P5 each of the four viruses were purified and the viral DNA extracted for analysis by the restriction digestion and radiolabeling procedure. This virus has subsequently been used in a series of studies (in vitro gag expression in COS cells, rodent study and rhesus monkey study) as will be described below. The viruses from P5 are shown in Figure 9.

The passaging under serum-free conditions was continued for the MRKHVE3 (transgene-less, obtained from MRKpAdHVE3 pre-plasmid) and the MRKAd5HIV-1gag (obtained from MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA pre-plasmid) viruses. Figure 10 shows viral DNA analysis by radioactive restriction digestion at passage 11 for MRKHVE3, MRKAd5HIV-1gagE3-, and passage 11 and 12 for MRKAd5HIV-1gag. Aside from the first lane which is the DNA marker lane, the next three lanes are virus from the pre-plasmid controls (controls based on the original virus) - MRKpAdHVE3 (also referred to as "pMRKHVE3"), MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA, and pMRKAd5gag(E3-), respectively. As seen in Figure 10, each of the viral DNA samples show the expected bands with no extraneous bands showing. This signifies that there are no major variant adenovirus species present that can be detected by autoradiography.

Figure 11 shows the results of viral competition study between MRKHVE3 and MRKAd5HIV-1gag. These viruses were mixed together at equal MOI (140 viral

particles each; 280 vp total) at passage 6 and continued to be passaged until P11. Aside from the first lane which is the DNA marker lane, the next two lanes are the pre-plasmid controls obtained from MRKpAdHVE3 and MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA. The next two lanes are the viral DNA from the starting viral material at passage six. The last two lanes are the competition studies performed in duplicate. The data in Figure 11 shows the effect the gag transgene in culture. Growth of a MRKAd5gag virus was compared with growth of a "transgene-less" MRKHVE3. These two viruses were infected at the same MOI (i.e. 140 vp each) at passage 6 and then passaged through to passage 11 and the viral pool was analyzed by radioactive restriction analysis. The data shows that one virus did not out compete the other. Therefore, the gag transgene did not show obvious signs of toxicity to the adenovirus.

5

10

15

20

25

30

Analysis by *Hind*III digestion shows that each virus specie is present in approximately equal amounts. As above, there does not appear to be signs of any extraneous bands. Figure 12 shows higher passage numbers for MRKAd5HIV-1gag grown under serum-containing conditions. The genome integrity again has been maintained and there is no evidence of rearrangements, even at the highest passage level (P21).

Each of the four vectors shown in Figure 9 were analyzed for amplification capacity. Table 4 below shows the QPA analysis used in the estimation of viral amplification ratios at P4. The determination of the amplification ratio for the original HIV-1 gag construct is based on the clinical lot at P12. It has been shown that amplification rates increases with higher passage number for the original virus. The reason for this observation is due to the emergence of variants which exhibit increased growth rates compared to the intact adenovector. With continued passaging of the original Ad gag vector, the level of variants increases and hence amplification rates increase also.

The MRK Ad5 HIV-1 gag virus has also been continually passaged under process conditions (i.e., serum-free media). Viral DNA extracted from passages 11 and 12 show no evidence of rearrangement.

Table 4:
Amplification Ratios Based on AEX and QPA Analysis of Virus Amplification from Passage 3 to Passage 4.

Ad gag construct	Amplification Ratio
MRKAd5gag	470
HCMV-Flgag-bGHpA [E3-]	115
HCMV-Flgag-SPA [E3+]	320
mCMV-FLgag-bGHpA [E3+]	420
Original construct *	40 - 50

5

EXAMPLE 13

10

15

20

25

Analytical Evaluation of the enhanced Ad5 Constructs

To study the effects of the transgene and the E3 gene on virus amplification, the enhanced adenoviral vector, MRK Ad5 HIV-1 gag, along with its transgene-less version (MRKpAdHVE3) and its E3- version (MRK Ad5 HIV-1 gag E3-), was studied for several passages under serum-free conditions. Table 5A shows the amplification ratios determined for passages P3 to P8 for MRK Ad5 HIV-1 gag. Within a certain MOI range, it has been determined that the virus output is directly proportional to the virus input. Therefore, the greater the number of virus particles per cell at infection, the greater the virus amount produced. Viral amplification ratios, on the other hand, are inversely proportional to the virus input. The lower the virus input, the greater the amplification ratio.

Table 5B shows the amplification rates of the new E3+ vector backbone MRKpAdHVE3. It has a significantly lower rate of amplification compared with the gag transgene containing version. This may be contributed to the larger size MRK Ad5 HIV-1 gag since it contains the transgene. This inclusion of the transgene brings the size of the adenovirus closer to the size of a wild type Ad5 virus. It is well known that adenoviruses amplify best when they are at close to their wild type genomic size.

^{*} This estimation is based on the clinical lot growth characteristics at Passage 12.

Wild type Ad5 is 35,935 bp. The MRKpAdHVE3 is 32, 905 bp in length. The enhanced adenovector MRK Ad5 HIV-1 gag is 35,453bp (See Figure 14 for vector map; see also Figure 15A-X show the complete pre-adenoviral vector sequence, which includes an additional 2,021 bp of the vector backbone).

5

10

Table 5C shows the amplification rates of the new E3- gag containing virus MRK Ad5 HIV-1 gag E3-. Once again, this virus shows lower growth rate than the enhanced adenoviral vector. This may be attributed to the decreased sized of this virus (due to the E3 gene deletion) compared with wild type Ad5. The MRK Ad5 HIV-1 gag E3- virus is 32,810 bp in length. This can be compared with the wild type Ad5 which is 35,935 bp and MRK Ad5 HIV-1 gag which is 35,453 bp in length.

Table 5A: Amplification ratios determined by AEX and QPA for **MRKAd5gag** over several continuous passaging in serum free media. Following P5, two replicate samples were taken (rep-1 and rep-2) and analyzed.

MRKAd5gag rep1

	Xv (10 calls/n	ni), Viability (%)	Harvest Time	Cell Passage	Titer	Titer	QPA	Ratio	Amplification	AEX
	Infection	Harvest	h.p.l.	Number	10 ¹⁰ vp/ml culture	10" vp/cell	10° TCID _{so} /mi	AEX:QPA	Ratto	Internal Control
P4	1.49, 81%	0.58, 50%	44	46	8.7	5.9	1.72	50	470 (MOI = 125)	
P5	1.38, 93%	0.66, 47%	48	49	6.7	4.9	1.38	49	170	
P6	1.04, 94%	0.68, 77%	47	48	5.8	5,6	1.42	41	200	
P7	1.50, 84%	0.96, 61%	49.5	50	3.9	1.4	0.97	40	50	
P7	1.09, 97%	0.78, 59%	50	52	5.2	4.7	1.70	31	170	
P8	1.03, 94%	0.88, 64%	47.5	54	9.0	8.7	1.10	62	310	
P9	0.89, 95%	0.99, 73%	47.5	56	4,4	4.9	1.03	43 .	175	3.12 2.84
P10	1.09, 91%	1.06, 66%	47,5	68	3.0	2.8	1.15	26	100	2.70 2.60
P11	1.19, 88%	0.98, 65%	47	60	3.6	3.0	1.15	31	110	2.70 2.70
P12	0.98, 91%	0.85, 63%	47.5	47	5.4	5.5	1.20	45	200	2,86 2,60
P13	1.00, 88%	0.70, 67%	49	49	5.8	5.8	1.11	52	210	3.18 3.18
P14	1.94, 92%	0.88, 67%	46	63	8.6	4.4			160	3.28 3.27
P15	0.97, 96%	0.64, 66%	47	47	6.9	7.1			250	3,12 2,91

Table 5B: Amplification ratios determined by AEX and QPA for **MRKHVE3** over several continuous passaging in serum free media. **MRKHVE3** is the new vector backbone which does NOT carry a transgene.

MRKHVE3

	Xv (10° calls/r	nl), Viability (%)	Harvest Time	Cell Passage	Titer .	Titer	QPA	Ratio	Amplification	AEX
	Infection	Harvest	hpi	Number	10 ¹⁰ vp/ml culture	10° vp/cell	10° TCID _{so} /ml	AEX:QPA	Ratio	Internal Control
P4	1.10, 97%	1.28, 79%	49	54	4.1	3.8	1.70	25	300 (MOI = 125)	
P5	0.92, 89%	1.18, 77%	47	. 48	4.3	4.7	1.24	35	170	
P6	1.55, 88%	1.28, 76%	49.5	50	1.2	0.8	0.56	21	30	
P6	1.09, 97%	1.11,81%	49	52	4.0	3.6	1.18	34	130	
P7	1.17, 91%	1.22, 91%	47.5	54	3.7	3.2	0.50	74	110	
P8	0.98, 88%	1.41, 63%	48	58	2.1	2.1	0.47	45	75	3.12 2.84
Pg	1.20, 89%	1.28, 81%	47,5	58	8,0	0.7	0.29	28	25	2.70 2.60
P10	0.99, 82%	1.55, 88%	47	60	2.3	2.3	0.43	53	80	2.70 2.70
P11	1.07, 98%	1.25, 83%	48	47	2.7	2.5	0.41	66	90	2.86 2.60
P12	0.80, 91%	1,14, 80%	49.5	49	5.9	7.4	0.48	123	260	3.18 3.18
P13	1.98, 95%	1.14, 85%	45.5	53	5.8	3.0			110	3.28 3.27
P14	0.97, 96%	1.03, 98%	48,5	47	9.4	9.7			350	3.12 2.91
P15	0.87, 99%	0.97, 59%	49.5	49	5.3	6.1			218	2.78 2.52

Table 5C. Amplification ratios determined by AEX and QPA for MRKAd5gag(E3-) over several continuous passaging in serum free media. This construct is identical to the MRKAd5gag construct except that this version is DELETED of the E3 gene.

5

MRKAd5qaq(E3-)

	Xv (10" cells/r	nl), Viability (%)	Harvest Time	Cell Passage	Titer	Titer	QPA	Ratio	Amplification	AEX
	Infection	Harvest	h.p.l.	Number	10 ¹⁰ vp/ml culture	10° vp/cell	10° TCID _{co} /ml	AEX;QPA	Ratio	internal Control
P4	1.62, 77%	1.12, 62%	47.5	46	2.0	1.2	0.92	20	100 (MOI=125)	
P6	1.16, 92%	0.62, 43%	49	49	3.3	2.9	0.99	34	100	
P6	1.71, 86%	0.20, 10%	49	50	4.7	2.7	1.70	28	100	
P6	1.09, 97%	0.63, 54%	49.5	52	5.4	5.0	1.76	31	180	
P7 .	1.17, 91%	0.98, 72%	47.50	54	7.1	6.1	0.67	106	220	
P8	0.98, 88%	0.77, 48%	48	56	3.1	3.2	0.66	47	115	3.12 2.84
P9	1.20, 89%	1.03, 72%	48	58	1,8	1.5	0.57	32	55	2.70 2.60
P10	0.99, 82%	0.80, 62%	46.5	60	3.2	3.2	0.68	47	115	2.70 2.70
P11	1.07, 95%	0.98, 70%	48.5	47	5.9	5.5	0.68	87	200	2.88 2.60
P12	0.80, 91%	0.67, 59%	50	49	5.1	6,4	0.72	71	230	3.18 3.18
P13	1.96, 95%	0.91, 59%	45.5	53	7.4	3.8			135	3.28 3.27
P14	0.97, 96%	0.81, 74%	48	47	6.8	7.0			250	3.12 2.91
P15	0.87, 99%	0.84, 56%	49	49	4.8	5.5			196	2.78 2.52

EXAMPLE 14

Gag Expression Analysis of the Novel Constructs

In vitro gag analysis of the MRK Ad5 HIV-1 gag and the original HIV-gag vectors (research and clinical lot) show comparable gag expression. The clinical lot shows only a slightly reduced gag expression level. The most noticeable difference is with the mCMV vector. This vector shows roughly 3 fold lower expression levels compared with the other vectors tested (which all contain hCMV promoters). The mCMV-FLgag with bGHpA assay was performed three times using different propagation and purification lots and it consistently exhibited weaker gag expression.

EXAMPLE 15

Evaluation of MRK Ad5 HIV-1 gag and Other gag-Containing Adenovectors in Balb/c Mice

Cohorts of 10 balb/c mice were vaccinated intramuscularly with escalating doses of MRK Ad5 HIV-1 gag, and the research and clinical lots of original Ad5HIV-1gag. Serum samples were collected 3 weeks post dose 1 and analyzed by anti-p24 sandwich ELISA.

Anti-p24 titers in mice that received MRK Ad5 HIV-1 gag (10⁷ and 10⁹ vp(viral particle) doses) were comparable (Figure 13) to those of the research lot of Ad5HIV-1 gag, for which much of the early rhesus data were generated on. These titers were also comparable when E3 is deleted (MRKAd5hCMVgagbGHpA(E3-)) or SPA is substituted for bGHpA terminator (MRKAd5 hCMV-gag-SPA (E3+)) or murine CMV promoter is used in place of hCMV (MRKAd5 mCMV-gag-bGHpA (E3+)) in the MRKAd5 backbone.

The results shown in Table 7 indicate that the three other vectors (in addition to the preferred vector, MRK Ad5 HIV-1 gag, are also capable of inducing strong anti-gag antibody responses in mice. Interestingly enough, while the mCMV-FLgag construct containing bGHpA and E3+ in an E1 parallel orientation showed lowest gag expression in the COS cell *in vitro* infection (Table 6) in comparison with the other vectors tested, it generated the greatest anti-gag antibody response this *in vivo* Balb/c study. Table 7 also shows a dose response in anti-gag antibody production in both the research and the clinical lot. As expected, the clinical lot shows reduced anti-gag antibody induction at each dosage level compared to the same dosage used for the research lot.

Table 6: In vitro analysis for gag expression in COS cells by Elisa assay.

20

10

15

Viral Vectors ^a	μg gag/4.8x10e5 COS/10e8 parts/48hr
MRKAd5gag ^b	1.40
Clinical lot Ad5gag ^c	1.28
Research lot Ad5gag ^d	1.32
MCMVFL-gagbGHpA ^c	0.42

^a A_{260nm} absorbance readings taken for viral particle determinations.

^b MRKAd5gag was produced in serum free conditions and purified at P5.

^c Clinical lot# Ad5gagFN0001

²⁵ d Research Ad5FLgag lot# 6399

e mCMVFL-gagbGHpA was produced in serum free conditions and purified at P5.

Table 7: mHIV020 Anti-p24 Ab Titers in Balb/c mice (n=10) vaccinated with various Adgag constructs and lots (3 week post dose1).

Vaccine	Dose	GMT	SE upper	SE lower
	(VP)			
^a MRKAd5gag	10^7	25600	5877	4780
n	10^9	409600	94028	76473
hCMV FL-gag bGHpA [E3-] →	10^7	7352	2077	1620
n .	10^9	235253	59767	47659
hCMV FL-gag SPA [E3+] →				236
' "	10^9	310419	99181	75165
bmoMVEL and bGHnA (E2.)	1007	44572	23504	15389
				190636
	10.0	541014	253000	130000
°hCMV FL-gag bGHpA [E3-] ←	10^7	3676	934	745
1 gag a stript time j.	10^9	117627	17491	15227
research lot hCMV intronA FL-gag bGHpA [E3-] <-	10^6	528		175
#	10^7	14703		3882
н .		58813	14942	11915
	10^9	204800	53232	42250
clinical lot hCMVintronA FL-gag bGHpA [E3-] <-				61
<u>"</u>				1138
				3274
	10^9	89144	2518/	19639
Naïva	none	93	7	6
	aMRKAd5gag hCMV FL-gag bGHpA [E3-] → hCMV FL-gag SPA [E3+] → bmCMV FL-gag bGHpA [E3+] → chCMV FL-gag bGHpA [E3-] ←	aMRKAd5gag 10^7 10^9 hCMV FL-gag bGHpA [E3-] → 10^7 10^9 hCMV FL-gag SPA [E3+] → 10^7 10^9 bmCMV FL-gag bGHpA [E3+] → 10^7 10^9 chCMV FL-gag bGHpA [E3-] ← 10^7 10^9 research lot hCMV intronA FL-gag bGHpA [E3-] <- 10^6 10^7 10^8 10^9 clinical lot hCMVintronA FL-gag bGHpA [E3-] <- 10^6 10^7 10^8 1009	amrkAd5gag 10^7 25600 409600 10^9 409600 hCMV FL-gag bGHpA [E3-] → 10^7 7352 235253 hCMV FL-gag SPA [E3+] → 10^7 12800 310419 hmCMV FL-gag bGHpA [E3+] → 10^7 44572 10^9 941014 hCMV FL-gag bGHpA [E3-] ← 10^7 3876 10^9 117627 research lot hCMV intronA FL-gag bGHpA [E3-] <- 10^6 528 10^7 14703 10^8 58813 10^9 204800 hCMV intronA FL-gag bGHpA [E3-] <- 10^6 230 10^7 4222 10^8 19401 10^9 89144	amrkAd5gag amrkAd5gag bGHpA [E3-] → bmCMV FL-gag bGHpA [E3+] → amrcMV FL-gag bGHpA [E3+] → bmCMV FL-gag bGHpA [E3+] → amrcMV FL-gag bGHpA [E3+] → bmCMV FL-gag bGHpA [E3+] → amrcMV FL-gag bGHpA [E3+] → amrcMV FL-gag bGHpA [E3-] ← amrcMV FL-gag bGHpA [E3-

*2x50 µL l.m. (quad) injections/animal P.l.s: Youil, Chen, Casimiro Vaccination: T. Toner, Q. Su

Assay: M. Chen

5

10

EXAMPLE 16

Comparison of Humoral and Cellular Responses Towards the Original Ad-gag Construct with the New MRK Ad5 HIV-1 gag in Rhesus Monkeys

Cohorts of 3 rhesus monkeys were vaccinated intramuscularly with MRK Ad5 HIV-1 gag or the clinical Ad5gag bulk at two doses, 10^{11} vp and 10^9 vp. Immunizations were conducted at week 0, 4, and 25. Serum and PBMC samples were collected at selected time points. The serum sample were assayed for anti-p24 Ab titers (using competitive based assay) and the PBMCs for antigen-specific IFN-gamma secretion following overnight stimulation with gag 20-mer peptide pool (via ELISpot assay).

The results shown in Table 8 indicate comparable responses with respect to the generation of anti-gag antibodies. The frequencies of gag-specific T cells in

^aThe structure of MRKAd5gag is: hCMVFL-gagbGHpA [E3+] → The <u>same lot</u> of MRKAd5gag used in this rodent study was used in the Rhesus monkey study (Tables 7 and 8).

^bThe same lot of mCMVFL-gagbGHpA[E3+] used in the in vitro study (Table 6) ws used here.

^cThis construct was designed by Volker Sandig. It contains a shorter version of the hCMV promoter than that used in the MRK constructs. The adenovector backbone is identical to the original backbone used in the original Adgag vector. Expression at 10e7 dose from this vector is 7 fold lower then the same dose of the MRKAd5gag and 4 fold lower than the research lot.

peripheral blood assummarized in Table 9 demonstrate a strong cellular immune response generated after a single dose with the new construct MRK Ad5 HIV-1 gag. The responses are also boostable with second dose of the same vector. The vector is also able to induce CD8+ T cell responses (as evident by remaining spot counts after CD4+ depletion of PBMCs) which are responsible for cytotoxic activity.

Table 8 Anti-p24 antibody titers (in mMU/mL) in rhesus macaques immunized with

gag-expressing adenovectors (Protocol HIV203).

Vaccine	Pre	Wk4	Wk8	Wk 12	Wk 16	Wk 20	Wk 25	Wk 28
MRKAd5gaga, 10^11 vp								
97N010	<10	118	5528	11523	7062	21997	ND	51593
97N116	<10	62	772	1447	1562	2174	ND	20029
98X007	<10	66	3353	6156	6845	3719	ND	24031
MRKAd5gag, 10^9 vp								
97N120	<10	51	204	318	366	482	ND	6550
97N144	<10	18	118	274	706	888	ND	7136
98X008	<10	15	444	386	996	1072	_ND	12851
Ad5gag ^b , Clinical Lot, 10^11 vp								
97X001	<10	87	2579	4718	7174	7250	ND	69226
97N146	<10	72	3604_	7380	7526	18906	ND	60283
98X009	<10	78	4183	3946	3124	6956	ND	26226
Ad5gag, Clinical Lot, 10^9 vp								
97N020	~10	<10	143	371	390	1821	ND	17177
97X003	<10	<10	39	93	156	596	Z	2053
98X012	<10	81	342	717	956	1558	DZ	11861
MRKAd5gag (hCMV, bGHpA, E3+)								
barlginal Adagagivector (hCMV/intra	A bGHp	A, E3-), lot#	FN0001					
ND, not determined				L				

5

Table 9. Number of gag-specific T cells per million peripheral blood mononuclear cells (PBMCs) in rhesus monkeys immunized with gag-expressing adenovectors. Also included are those frequencies in PBMCs depleted of CD4⁺ T cells.

Grp #	Vaccination	Monkey ID	I=4	Wk	I =6	Wk	T=1	l Wk		6 Wk	T=2	5 Wk	T=2	8 Wk
•••	T=0,4,25 wks		Media	Gog H ^b	Media	Gog H	Media	Gog H	Media	Gog H	Media	Gog H	Media	Gog H
1	MRKAcāgag 10^11 vp	97N010 97N010(CO4-)	6 4	89 38 396	0	395 609	0 3 0	1058 993 534	0	1174 395	3 0	775 76 261	4 0 0	1074 594 408
		97N116 97N116(CD4-) 98X007 98X007(CD4-)	1 11 10 20	676 579 965	0	1304	0 3 0	593 2193 2675	1	2118	0 3 0	184 1588 1656	0	666 2113 1278
2	MRKAc5gcg 10·9 vp	97N120 97N120(CD4-) 97N144 97N144(CD4-) 98X008 98X008(CD4-)	5 11 3 6 4	275 170 236 148 368 696	1 6 1	249 438 1090	4 0 1 0 3	141 85 318 285 891 1175	4 3 4	119 256 673	001 <u>9</u> 30	206 75 98 ND 473 391	4 1 5 0 5 4	219 219 373 625 735 848
3	AdSpag dintral lat 10^11 vp	97X001 97X001(CD4-) 97N146 97N146(CD4-) 98X009 98X009(CD4-)	0 10 3 6 0	261 283 150 133 93 73	1 3	485 485 339	0 3 0 0 3	817 996 339 370 559 333	0 1 0	1220b 1272 896	1 0 3 0 1	894 1010 1238 654 384 225	0 3 0 0	1858 1123 1785 971 1748 644
4	Acāgag cliniad lai 1049 vp	97N020 97N020(CD4-) 97X003 97X003(CD4-) 98X012 98X012(CD4-)	3 10 4 9 5	30 29 68 40 95 70	1 5 3	101 134 54	0 0 0 1 0	66 15 18 6 34 11	0 1 0	36 38 18	004000	26 1 38 4 20 8	006010	41 16 81 19 121 41
5	Ndve	96R041 053F	6 14	8 18	1 5	1 18.	0 20	0 14	D 19	0 15	0 10	0 15	1 24	80

Based on either 4x10/6 or 2x10/6 cells per well (depending on spot density)

ND, not determined

5

10

15

20

The adenovectors described herein and, particularly, MRK Ad5 HIV-1 gag, represent very promising HIV-gag adenovectors with respect to their enhanced growth characteristics in both serum and, more importantly, in serum-free media conditions. In comparison with the current HIV-1 gag adenovector construct, MRK Ad5 HIV-1 gag shows a 5-10 fold increased amplification rate. We have shown that it is genetically stable at passage 21. This construct is able to generate significant cellular immune responses *in vivo* even at a relatively low dose of 10^9 vp. The potency of the MRKAd5gag construct is comparable to, if not better than the original HIV-1gag vector as shown in this rhesus monkey study.

EXAMPLE 17 CODON OPTIMIZED HIV-1 POL AND CODON OPTIMZED HIV-1 POL MODIFICATIONS

The open reading frames for the various synthetic *pol* genes disclosed herein comprise coding sequences for the reverse transcriptase (or RT which consists of a polymerase and RNase H activity) and integrase (IN). The protein sequence is based

Pod of 20-capepitoes overlanding by 10 as and encompassing the pagsequence

on that of Hxb2r, a clonal isolate of IIIB; this sequence has been shown to be closest to the consensus clade B sequence with only 16 nonidentical residues out of 848 (Korber, et al., 1998, Human retroviruses and AIDS, Los Alamos National Laboratory, Los Alamos, New Mexico). The skilled artisan will understand after review of this specification that any available HIV-1 or HIV-2 strain provides a potential template for the generation of HIV pol DNA vaccine constructs disclosed herein. It is further noted that the protease gene is excluded from the DNA vaccine constructs of the present invention to insure safety from any residual protease activity in spite of mutational inactivation. The design of the gene sequences for both wildtype (wt-pol) and inactivated pol (IA-pol) incorporates the use of human preferred ("humanized") codons for each amino acid residue in the sequence in order to maximize in vivo mammalian expression (Lathe, 1985, J. Mol. Biol. 183:1-12). As can be discerned by inspecting the codon usage in SEQ ID NOs: 1, 3, 5 and 7, the following codon usage for mammalian optimization is preferred: Met (ATG), Gly (GGC), Lys (AAG), Trp (TGG), Ser (TCC), Arg (AGG), Val (GTG), Pro (CCC), Thr (ACC), Glu (GAG); Leu (CTG), His (CAC), Ile (ATC), Asn (AAC), Cys (TGC), Ala (GCC), Gln (CAG), Phe (TTC) and Tyr (TAC). For an additional discussion relating to mammalian (human) codon optimization, see WO 97/31115 (PCT/US97/02294), which, as noted elsewhere in this specification, is hereby incorporated by reference. It is intended that the skilled artisan may use alternative versions of codon optimization or may omit this step when generating HIV pol vaccine constructs within the scope of the present invention. Therefore, the present invention also relates to non-codon optimized versions of DNA molecules and associated recombinant adenoviral HIV vaccines which encode the various wild type and modified forms of the HIV Pol protein disclosed herein. However, codon optimization of these constructs is a preferred embodiment of this invention.

10

15

20

25

30

35

A particular embodiment of this portion of the invention comprisies codon optimized nucleotide sequences which encode wt-pol DNA constructs (herein, "wt-pol" or "wt-pol (codon optimized))" wherein DNA sequences encoding the protease (PR) activity are deleted, leaving codon optimized "wild type" sequences which encode RT (reverse transcriptase and RNase H activity) and IN integrase activity. A DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:1, the open reading frame being contained from an initiating Met residue at nucleotides 10-12 to a termination codon from nucleotides 2560-2562. SEQ ID NO:1 is as follows:

AGATCTACCA TGGCCCCCAT CTCCCCCATT GAGACTGTGC CTGTGAAGCT GAAGCCTGGC

ATGGATGGCC CCAAGGTGAA GCAGTGGCCC CTGACTGAGG AGAAGATCAA GGCCCTGGTG

	GAAATCTGCA	CTGAGATGGA	GAAGGAGGC	AAAATCTCCA	AGATTGGCCC	CGAGAACCCC
	TACAACACCC	CTGTGTTTGC	CATCAAGAAG	AAGGACTCCA	CCAAGTGGAG	GAAGCTGGTG
	GACTTCAGGG	AGCTGAACAA	GAGGACCCAG	GACTTCTGGG	AGGTGCAGCT	GGGCATCCCC
	CACCCCGCTG	GCCTGAAGAA	GAAGAAGTCT	GTGACTGTGC	TGGATGTGGG	GGATGCCTAC
5	TTCTCTGTGC	CCCTGGATGA	GGACTTCAGG	AAGTACACTG	CCTTCACCAT	CCCCTCCATC
	AACAATGAGA	CCCCTGGCAT	CAGGTACCAG	TACAATGTGC	TGCCCCAGGG	CTGGAAGGGC
	TCCCCTGCCA	TCTTCCAGTC	CTCCATGACC	AAGATCCTGG	AGCCCTTCAG	GAAGCAGAAC
	CCTGACATTG	TGATCTACCA	GTACATGGAT	GACCTGTATG	TGGGCTCTGA	CCTGGAGATT
	GGGCAGCACA	GGACCAAGAT	TGAGGAGCTG	AGGCAGCACC	TGCTGAGGTG	GGGCCTGACC
10	ACCCCTGACA	AGAAGCACCA	GAAGGAGCCC	CCCTTCCTGT	GGATGGGCTA	TGAGCTGCAC
	CCCGACAAGT	GGACTGTGCA	GCCCATTGTG	CTGCCTGAGA	AGGACTCCTG	GACTGTGAAT
	GACATCCAGA	AGCTGGTGGG	CAAGCTGAAC	TGGGCCTCCC	AAATCTACCC	TGGCATCAAG
	GTGAGGCAGC	TGTGCAAGCT	GCTGAGGGGC	ACCAAGGCCC	TGACTGAGGT	GATCCCCCTG
	ACTGAGGAGG	CTGAGCTGGA	GCTGGCTGAG	AACAGGGAGA	TCCTGAAGGA	GCCTGTGCAT
15	GGGGTGTACT	ATGACCCCTC	CAAGGACCTG	ATTGCTGAGA	TCCAGAAGCA	GGGCCAGGGC
	CAGTGGACCT	ACCAAATCTA	CCAGGAGCCC	TTCAAGAACC	TGAAGACTGG	CAAGTATGCC
	AGGATGAGGG	GGGCCCACAC	CAATGATGTG	AAGCAGCTGA	CTGAGGCTGT	GCAGAAGATC
	ACCACTGAGT	CCATTGTGAT	CTGGGGCAAG	ACCCCCAAGT	TCAAGCTGCC	CATCCAGAAG
	GAGACCTGGG	AGACCTGGTG	GACTGAGTAC	TGGCAGGCCA	CCTGGATCCC	TGAGTGGGAG
20	TTTGTGAACA	CCCCCCCCT	GGTGAAGCTG	TGGTACCAGC	TGGAGAAGGA	GCCCATTGTG
	GGGGCTGAGA	CCTTCTATGT	GGATGGGGCT	GCCAACAGGG	AGACCAAGCT	GGGCAAGGCT
	GGCTATGTGA	CCAACAGGGG	CAGGCAGAAG	GTGGTGACCC	TGACTGACAC	CACCAACCAG
	AAGACTGAGC	TCCAGGCCAT	CTACCTGGCC	CTCCAGGACT	CTGGCCTGGA	GGTGAACATT
	GTGACTGACT	CCCAGTATGC	CCTGGGCATC	ATCCAGGCCC	AGCCTGATCA	GTCTGAGTCT
25	GAGCTGGTGA	ACCAGATCAT	TGAGCAGCTG	ATCAAGAAGG	AGAAGGTGTA	CCTGGCCTGG
	GTGCCTGCCC	ACAAGGGCAT	TGGGGGCAAT	GAGCAGGTGG	ACAAGCTGGT	GTCTGCTGGC
	ATCAGGAAGG	TGCTGTTCCT	GGATGGCATT	GACAAGGCCC	AGGATGAGCA	TGAGAAGTAC
	CACTCCAACT	GGAGGGCTAT	GGCCTCTGAC	TTCAACCTGC	CCCCTGTGGT	GGCTAAGGAG
	ATTGTGGCCT	CCTGTGACAA	GTGCCAGCTG	AAGGGGGAGG	CCATGCATGG	GCAGGTGGAC
30	TGCTCCCCTG	GCATCTGGCA	GCTGGACTGC	ACCCACCTGG	AGGGCAAGGT	GATCCTGGTG
,	GCTGTGCATG	TGGCCTCCGG	CTACATTGAG	GCTGAGGTGA	TCCCTGCTGA	GACAGGCCAG
	GAGACTGCCT	ACTTCCTGCT	GAAGCTGGCT	GGCAGGTGGC	CTGTGAAGAC	CATCCACACT
	GACAATGGCT	CCAACTTCAC	TGGGGCCACA	GTGAGGGCTG	CCTGCTGGTG	GGCTGGCATC
	AAGCAGGAGT	TTGGCATCCC	CTACAACCCC	CAGTCCCAGG	GGGTGGTGGA	GTCCATGAAC
35	AAGGAGCTGA	AGAAGATCAT	TGGGCAGGTG	AGGGACCAGG	CTGAGCACCT	GAAGACAGCT
	GTGCAGATGG	CTGTGTTCAT	CCACAACTTC	AAGAGGAAGG	GGGGCATCGG	GGGCTACTCC

GCTGGGGAGA GGATTGTGGA CATCATTGCC ACAGACATCC AGACCAAGGA GCTCCAGAAG
CAGATCACCA AGATCCAGAA CTTCAGGGTG TACTACAGGG ACTCCAGGAA CCCCCTGTGG
AAGGGCCCTG CCAAGCTGCT GTGGAAGGGG GAGGGGGCTG TGGTGATCCA GGACAACTCT
GACATCAAGG TGGTGCCCAG GAGGAAGGCC AAGATCATCA GGGACTATGG CAAGCAGATG
GCTGGGGATG ACTGTGTGGC CTCCAGGCAG GATGAGGACT AAAGCCCGGG CAGATCT (SEQ
ID NO:1).

The open reading frame of the wild type pol construct disclosed as SEO ID NO:1 contains 850 amino acids, disclosed herein as SEQ ID NO:2, as follows: Met Ala Pro Ile Ser Pro Ile Glu Thr Val Pro Val Lys Leu Lys Pro 10 Gly Met Asp Gly Pro Lys Val Lys Gln Trp Pro Leu Thr Glu Glu Lys Ile Lys Ala Leu Val Glu Ile Cys Thr Glu Met Glu Lys Glu Gly Lys Ile Ser Lys Ile Gly Pro Glu Asn Pro Tyr Asn Thr Pro Val Phe Ala Ile Lys Lys Lys Asp Ser Thr Lys Trp Arg Lys Leu Val Asp Phe Arg Glu Leu Asn Lys Arg Thr Gln Asp Phe Trp Glu Val Gln Leu Gly Ile 15 Pro His Pro Ala Gly Leu Lys Lys Lys Lys Ser Val Thr Val Leu Asp Val Gly Asp Ala Tyr Phe Ser Val Pro Leu Asp Glu Asp Phe Arg Lys Tyr Thr Ala Phe Thr Ile Pro Ser Ile Asn Asn Glu Thr Pro Gly Ile Arg Tyr Gln Tyr Asn Val Leu Pro Gln Gly Trp Lys Gly Ser Pro Ala Ile Phe Gln Ser Ser Met Thr Lys Ile Leu Glu Pro Phe Arg Lys Gln 20 Asn Pro Asp Ile Val Ile Tyr Gln Tyr Met Asp Asp Leu Tyr Val Gly Ser Asp Leu Glu Ile Gly Gln His Arg Thr Lys Ile Glu Glu Leu Arg Gln His Leu Leu Arg Trp Gly Leu Thr Thr Pro Asp Lys Lys His Gln Lys Glu Pro Pro Phe Leu Trp Met Gly Tyr Glu Leu His Pro Asp Lys Trp Thr Val Gln Pro Ile Val Leu Pro Glu Lys Asp Ser Trp Thr Val 25 Asn Asp Ile Gln Lys Leu Val Gly Lys Leu Asn Trp Ala Ser Gln Ile Tyr Pro Gly Ile Lys Val Arg Gln Leu Cys Lys Leu Leu Arg Gly Thr Lys Ala Leu Thr Glu Val Ile Pro Leu Thr Glu Glu Ala Glu Leu Glu Leu Ala Glu Asn Arg Glu Ile Leu Lys Glu Pro Val His Gly Val Tyr Tyr Asp Pro Ser Lys Asp Leu Ile Ala Glu Ile Gln Lys Gln Gly Gln 30 Gly Gln Trp Thr Tyr Gln Ile Tyr Gln Glu Pro Phe Lys Asn Leu Lys Thr Gly Lys Tyr Ala Arg Met Arg Gly Ala His Thr Asn Asp Val Lys Gln Leu Thr Glu Ala Val Gln Lys Ile Thr Thr Glu Ser Ile Val Ile Trp Gly Lys Thr Pro Lys Phe Lys Leu Pro Ile Gln Lys Glu Thr Trp Glu Thr Trp Trp Thr Glu Tyr Trp Gln Ala Thr Trp Ile Pro Glu Trp 35 Glu Phe Val Asn Thr Pro Pro Leu Val Lys Leu Trp Tyr Gln Leu Glu Lys Glu Pro Ile Val Gly Ala Glu Thr Phe Tyr Val Asp Gly Ala Ala

Asn Arg Glu Thr Lys Leu Gly Lys Ala Gly Tyr Val Thr Asn Arg Gly Arg Gln Lys Val Val Thr Leu Thr Asp Thr Thr Asn Gln Lys Thr Glu Leu Gln Ala Ile Tyr Leu Ala Leu Gln Asp Ser Gly Leu Glu Val Asn Ile Val Thr Asp Ser Gln Tyr Ala Leu Gly Ile Ile Gln Ala Gln Pro Asp Gln Ser Glu Ser Glu Leu Val Asn Gln Ile Ile Glu Gln Leu Ile Lys Lys Glu Lys Val Tyr Leu Ala Trp Val Pro Ala His Lys Gly Ile Gly Gly Asn Glu Gln Val Asp Lys Leu Val Ser Ala Gly Ile Arg Lys Val Leu Phe Leu Asp Gly Ile Asp Lys Ala Gln Asp Glu His Glu Lys Tyr His Ser Asn Trp Arg Ala Met Ala Ser Asp Phe Asn Leu Pro Pro Val Val Ala Lys Glu Ile Val Ala Ser Cys Asp Lys Cys Gln Leu Lys 10 Gly Glu Ala Met His Gly Gln Val Asp Cys Ser Pro Gly Ile Trp Gln Leu Asp Cys Thr His Leu Glu Gly Lys Val Ile Leu Val Ala Val His Val Ala Ser Gly Tyr Ile Glu Ala Glu Val Ile Pro Ala Glu Thr Gly Gln Glu Thr Ala Tyr Phe Leu Leu Lys Leu Ala Gly Arg Trp Pro Val 15 Lys Thr Ile His Thr Asp Asn Gly Ser Asn Phe Thr Gly Ala Thr Val Arg Ala Ala Cys Trp Trp Ala Gly Ile Lys Gln Glu Phe Gly Ile Pro Tyr Asn Pro Gln Ser Gln Gly Val Val Glu Ser Met Asn Lys Glu Leu Lys Lys Ile Ile Gly Gln Val Arg Asp Gln Ala Glu His Leu Lys Thr Ala Val Gln Met Ala Val Phe Ile His Asn Phe Lys Arg Lys Gly Gly 20 Ile Gly Gly Tyr Ser Ala Gly Glu Arg Ile Val Asp Ile Ile Ala Thr Asp Ile Gln Thr Lys Glu Leu Gln Lys Gln Ile Thr Lys Ile Gln Asn Phe Arg Val Tyr Tyr Arg Asp Ser Arg Asn Pro Leu Trp Lys Gly Pro Ala Lys Leu Trp Lys Gly Glu Gly Ala Val Val Ile Gln Asp Asn Ser Asp Ile Lys Val Val Pro Arg Arg Lys Ala Lys Ile Ile Arg Asp 25 Tyr Gly Lys Gln Met Ala Gly Asp Asp Cys Val Ala Ser Arg Gln Asp Glu Asp (SEQ ID NO:2).

The present invention especially relates to an adenoviral vector vaccine which comprises a codon optimized HIV-1 DNA pol construct wherein, in addition to deletion of the portion of the wild type sequence encoding the protease activity, a combination of active site residue mutations are introduced which are deleterious to HIV-1 pol (RT-RH-IN) activity of the expressed protein. Therefore, the present invention preferably relates to an adenoviral HIV-1 DNA pol-based vaccine wherein the construct is devoid of DNA sequences encoding any PR activity, as well as containing a mutation(s) which at least partially, and preferably substantially, abolishes RT, RNase and/or IN activity. One type of HIV-1 pol mutant which is part and parcel of an adenoviral vector vaccine may include but is not limited to a mutated

30

35

DNA molecule comprising at least one nucleotide substitution which results in a point mutation which effectively alters an active site within the RT, RNase and/or IN regions of the expressed protein, resulting in at least substantially decreased enzymatic activity for the RT, RNase H and/or IN functions of HIV-1 Pol. In a preferred embodiment of this portion of the invention, a HIV-1 DNA pol construct contains a mutation or mutations within the Pol coding region which effectively abolishes RT, RNase H and IN activity. An especially preferable HIV-1 DNA pol construct in a DNA molecule which contains at least one point mutation which alters the active site of the RT, RNase H and IN domains of Pol, such that each activity is at least substantially abolished. Such a HIV-1 Pol mutant will most likely comprise at least one point mutation in or around each catalytic domain responsible for RT, RNase H and IN activity, respectfully. To this end, an especially preferred HIV-1 DNA pol construct is exemplified herein and contains nine codon substitution mutations which results in an inactivated Pol protein (IA Pol: SEO ID NO:4, Figure 17A-C) which has no PR, RT, RNase or IN activity, wherein three such point mutations reside within each of the RT, RNase and IN catalytic domains. Therefore, an especially preferred exemplification is an adenoviral vaccine which comprises, in an appropriate fashion, a DNA molecule which encodes IA-pol, which contains all nine mutations as shown below in Table 1. An additional preferred amino acid residue for substitution is Asp551, localized within the RNase domain of Pol. Any combination of the mutations disclosed herein may suitable and therefore may be utilized as an IA-Pol-based vaccine of the present invention. While addition and deletion mutations are contemplated and within the scope of the invention, the preferred mutation is a point mutation resulting in a substitution of the wild type amino acid with an alternative amino acid residue.

10

15

20

25

•			Table 1	
	wt aa	aa residue	mutant aa	enzyme function
	Asp	112	Ala	RT
	Asp	187	Ala	RT
30	Asp	188	Ala	RT
	Asp	445	Ala	RNase H
	Glu	480	Ala	RNase H
	Asp	500	Ala	RNase H
	Asp	626	Ala	IN
35	Asp	678	Ala	IN
	Glu	714	Ala	IN

It is preferred that point mutations be incorporated into the IApol mutant adenoviral vaccines of the present invention so as to lessen the possibility of altering epitopes in and around the active site(s) of HIV-1 Pol.

To this end, SEQ ID NO:3 discloses the nucleotide sequence which codes for a codon optimized pol in addition to the nine mutations shown in Table 1, disclosed as follows, and referred to herein as "IApol":

5

AGATCTACCA TGGCCCCCAT CTCCCCCATT GAGACTGTGC CTGTGAAGCT GAAGCCTGGC ATGGATGGCC CCAAGGTGAA GCAGTGGCCC CTGACTGAGG AGAAGATCAA GGCCCTGGTG GAAATCTGCA CTGAGATGGA GAAGGAGGGC AAAATCTCCA AGATTGGCCC CGAGAACCCC TACAACACCC CTGTGTTTGC CATCAAGAAG AAGGACTCCA CCAAGTGGAG GAAGCTGGTG 10 GACTTCAGGG AGCTGAACAA GAGGACCCAG GACTTCTGGG AGGTGCAGCT GGGCATCCCC CACCCCGCTG GCCTGAAGAA GAAGAAGTCT GTGACTGTGC TGGCTGTGGG GGATGCCTAC TTCTCTGTGC CCCTGGATGA GGACTTCAGG AAGTACACTG CCTTCACCAT CCCCTCCATC AACAATGAGA CCCCTGGCAT CAGGTACCAG TACAATGTGC TGCCCCAGGG CTGGAAGGGC TCCCCTGCCA TCTTCCAGTC CTCCATGACC AAGATCCTGG AGCCCTTCAG GAAGCAGAAC 15 CCTGACATTG TGATCTACCA GTACATGGCT GCCCTGTATG TGGGCTCTGA CCTGGAGATT GGGCAGCACA GGACCAAGAT TGAGGAGCTG AGGCAGCACC TGCTGAGGTG GGGCCTGACC ACCCCTGACA AGAAGCACCA GAAGGAGCCC CCCTTCCTGT GGATGGGCTA TGAGCTGCAC CCCGACAAGT GGACTGTGCA GCCCATTGTG CTGCCTGAGA AGGACTCCTG GACTGTGAAT GACATCCAGA AGCTGGTGGG CAAGCTGAAC TGGGCCTCCC AAATCTACCC TGGCATCAAG 20 GTGAGGCAGC TGTGCAAGCT GCTGAGGGGC ACCAAGGCCC TGACTGAGGT GATCCCCCTG ACTGAGGAGG CTGAGCTGGA GCTGGCTGAG AACAGGGAGA TCCTGAAGGA GCCTGTGCAT GGGGTGTACT ATGACCCCTC CAAGGACCTG ATTGCTGAGA TCCAGAAGCA GGGCCAGGGC CAGTGGACCT ACCAAATCTA CCAGGAGCCC TTCAAGAACC TGAAGACTGG CAAGTATGCC AGGATGAGGG GGGCCCACAC CAATGATGTG AAGCAGCTGA CTGAGGCTGT GCAGAAGATC 25 ACCACTGAGT CCATTGTGAT CTGGGGCAAG ACCCCCAAGT TCAAGCTGCC CATCCAGAAG GAGACCTGGG AGACCTGGTG GACTGAGTAC TGGCAGGCCA CCTGGATCCC TGAGTGGGAG TTTGTGAACA CCCCCCCCT GGTGAAGCTG TGGTACCAGC TGGAGAAGGA GCCCATTGTG GGGGCTGAGA CCTTCTATGT GGCTGGGGCT GCCAACAGGG AGACCAAGCT GGGCAAGGCT 30 GGCTATGTGA CCAACAGGGG CAGGCAGAAG GTGGTGACCC TGACTGACAC CACCAACCAG AAGACTGCCC TCCAGGCCAT CTACCTGGCC CTCCAGGACT CTGGCCTGGA GGTGAACATT GTGACTGCCT CCCAGTATGC CCTGGGCATC ATCCAGGCCC AGCCTGATCA GTCTGAGTCT GTGCCTGCCC ACAAGGGCAT TGGGGGCAAT GAGCAGGTGG ACAAGCTGGT GTCTGCTGGC ATCAGGAAGG TGCTGTTCCT GGATGGCATT GACAAGGCCC AGGATGAGCA TGAGAAGTAC 35 CACTCCAACT GGAGGGCTAT GGCCTCTGAC TTCAACCTGC CCCCTGTGGT GGCTAAGGAG

ATTGTGGCCT CCTGTGACAA GTGCCAGCTG AAGGGGGAGG CCATGCATGG GCAGGTGGAC
TGCTCCCCTG GCATCTGGCA GCTGGCCTGC ACCCACCTG AGGGCAAGGT GATCCTGGTG
GCTGTGCATG TGGCCTCCGG CTACATTGAG GCTGAGGTGA TCCCTGCTGA GACAAGGCCAG
GAGACTGCCT ACTTCCTGCT GAAGCTGGCT GGCAGGTGGC CTGTGAAGAC CATCCACACT
GCCAATGGCT CCAACTTCAC TGGGGCCACA GTGAGGGCTG CCTGCTGGTG GGCTGGCATC
AAGCAGGAGT TTGGCATCCC CTACAACCCC CAGTCCCAGG GGGTGGTGGC CTCCATGAAC
AAGGAGCTGA AGAAGATCAT TGGGCAGGTG AGGGACCAGG GGGGCATCG GAAGACAGCT
GTGCAGATGG CTGTGTTCAT CCACAACTTC AAGAGGAAGG GGGGCATCGG GGGCTACTCC
GCTGGGGAGA GGATTGTGGA CATCATTGCC ACAGACATC AGACCAAGGA GCTCCAGAAG
CAGATCACCA AGATCCAGAA CTTCAGGGTG TACTACAGGG ACTCCAGGAA CCCCCTGTGG
AAGGGCCCTG CCAAGCTGCT GTGGAAGGGG GAGGGGCCTG TGGTGATCCA GGACAACTCT
GACATCAAGG TGGTGCCCAG GAGGAAGGCC AAGATCATCA GGGACTATGG CAAGCAGATG
GCTGGGGATG ACTGTGGGC CTCCAGGAG GATGAGGACT AAAGCCCGGG CAGATCT (SEQ ID
NO:3).

5

10

15

20

25

30

35

In order to produce the IA-pol-based adenoviral vaccines of the present invention, inactivation of the enzymatic functions was achieved by replacing a total of nine active site residues from the enzyme subunits with alanine side-chains. As shown in Table 1, all residues that comprise the catalytic triad of the polymerase, namely Asp112, Asp187, and Asp188, were substituted with alanine (Ala) residues (Larder, et al., Nature 1987, 327: 716-717; Larder, et al., 1989, Proc. Natl. Acad. Sci. 1989, 86: 4803-4807). Three additional mutations were introduced at Asp445, Glu480 and Asp500 to abolish RNase H activity (Asp551 was left unchanged in this IA Pol construct), with each residue being substituted for an Ala residue, respectively (Davies, et al., 1991, Science 252:, 88-95; Schatz, et al., 1989, FEBS Lett. 257: 311-314; Mizrahi, et al., 1990, Nucl. Acids. Res. 18: pp. 5359-5353). HIV pol integrase function was abolished through three mutations at Asp626, Asp678 and Glu714. Again, each of these residues has been substituted with an Ala residue (Wiskerchen, et al., 1995, J. Virol. 69: 376-386; Leavitt, et al., 1993, J. Biol. Chem. 268: 2113-2119). Amino acid residue Pro3 of SEQ ID NO:4 marks the start of the RT gene. The complete amino acid sequence of IA-Pol is disclosed herein as SEQ ID NO:4 and Figure 17A-C, as follows:

Met Ala Pro Ile Ser Pro Ile Glu Thr Val Pro Val Lys Leu Lys Pro Gly Met Asp Gly Pro Lys Val Lys Gln Trp Pro Leu Thr Glu Glu Lys Ile Lys Ala Leu Val Glu Ile Cys Thr Glu Met Glu Lys Glu Gly Lys Ile Ser Lys Ile Gly Pro Glu Asn Pro Tyr Asn Thr Pro Val Phe Ala Ile Lys Lys Lys Lys Asp Ser Thr Lys Trp Arg Lys Leu Val Asp Phe Arg

Glu Leu Asn Lys Arg Thr Gln Asp Phe Trp Glu Val Gln Leu Gly Ile Pro His Pro Ala Gly Leu Lys Lys Lys Ser Val Thr Val Leu Ala Val Gly Asp Ala Tyr Phe Ser Val Pro Leu Asp Glu Asp Phe Arg Lys Tyr Thr Ala Phe Thr Ile Pro Ser Ile Asn Asn Glu Thr Pro Gly Ile Arg Tyr Gln Tyr Asn Val Leu Pro Gln Gly Trp Lys Gly Ser Pro Ala Ile Phe Gln Ser Ser Met Thr Lys Ile Leu Glu Pro Phe Arg Lys Gln Asn Pro Asp Ile Val Ile Tyr Gln Tyr Met Ala Ala Leu Tyr Val Gly Ser Asp Leu Glu Ile Gly Gln His Arg Thr Lys Ile Glu Glu Leu Arg Gln His Leu Leu Arg Trp Gly Leu Thr Thr Pro Asp Lys Lys His Gln Lys Glu Pro Pro Phe Leu Trp Met Gly Tyr Glu Leu His Pro Asp Lys 10 Trp Thr Val Gln Pro Ile Val Leu Pro Glu Lys Asp Ser Trp Thr Val Asn Asp Ile Gln Lys Leu Val Gly Lys Leu Asn Trp Ala Ser Gln Ile Tyr Pro Gly Ile Lys Val Arg Gln Leu Cys Lys Leu Leu Arg Gly Thr Lys Ala Leu Thr Glu Val Ile Pro Leu Thr Glu Glu Ala Glu Leu Glu Leu Ala Glu Asn Arg Glu Ile Leu Lys Glu Pro Val His Gly Val Tyr 15 Tyr Asp Pro Ser Lys Asp Leu Ile Ala Glu Ile Gln Lys Gln Gly Gln Gly Gln Trp Thr Tyr Gln Ile Tyr Gln Glu Pro Phe Lys Asn Leu Lys Thr Gly Lys Tyr Ala Arg Met Arg Gly Ala His Thr Asn Asp Val Lys Gln Leu Thr Glu Ala Val Gln Lys Ile Thr Thr Glu Ser Ile Val Ile 20 Trp Gly Lys Thr Pro Lys Phe Lys Leu Pro Ile Gln Lys Glu Thr Trp Glu Thr Trp Trp Thr Glu Tyr Trp Gln Ala Thr Trp Ile Pro Glu Trp Glu Phe Val Asn Thr Pro Pro Leu Val Lys Leu Trp Tyr Gln Leu Glu Lys Glu Pro Ile Val Gly Ala Glu Thr Phe Tyr Val Ala Gly Ala Ala Asn Arg Glu Thr Lys Leu Gly Lys Ala Gly Tyr Val Thr Asn Arg Gly Arg Gln Lys Val Val Thr Leu Thr Asp Thr Thr Asn Gln Lys Thr Ala 25 Leu Gln Ala Ile Tyr Leu Ala Leu Gln Asp Ser Gly Leu Glu Val Asn Ile Val Thr Ala Ser Gln Tyr Ala Leu Gly Ile Ile Gln Ala Gln Pro Asp Gln Ser Glu Ser Glu Leu Val Asn Gln Ile Ile Glu Gln Leu Ile Lys Lys Glu Lys Val Tyr Leu Ala Trp Val Pro Ala His Lys Gly Ile Gly Gly Asn Glu Gln Val Asp Lys Leu Val Ser Ala Gly Ile Arg Lys 30 · Val Leu Phe Leu Asp Gly Ile Asp Lys Ala Gln Asp Glu His Glu Lys Tyr His Ser Asn Trp Arg Ala Met Ala Ser Asp Phe Asn Leu Pro Pro Val Val Ala Lys Glu Ile Val Ala Ser Cys Asp Lys Cys Gln Leu Lys Gly Glu Ala Met His Gly Gln Val Asp Cys Ser Pro Gly Ile Trp Gln Leu Ala Cys Thr His Leu Glu Gly Lys Val Ile Leu Val Ala Val His 35 Val Ala Ser Gly Tyr Ile Glu Ala Glu Val Ile Pro Ala Glu Thr Gly

Gln Glu Thr Ala Tyr Phe Leu Leu Lys Leu Ala Gly Arg Trp Pro Val Lys Thr Ile His Thr Ala Asn Gly Ser Asn Phe Thr Gly Ala Thr Val Arg Ala Ala Cys Trp Trp Ala Gly Ile Lys Gln Glu Phe Gly Ile Pro Tyr Asn Pro Gln Ser Gln Gly Val Val Ala Ser Met Asn Lys Glu Leu Lys Lys Ile Ile Gly Gln Val Arg Asp Gln Ala Glu His Leu Lys Thr Ala Val Gln Met Ala Val Phe Ile His Asn Phe Lys Arg Lys Gly Gly Ile Gly Gly Tyr Ser Ala Gly Glu Arg Ile Val Asp Ile Ile Ala Thr Asp Ile Gln Thr Lys Glu Leu Gln Lys Gln Ile Thr Lys Ile Gln Asn Phe Arg Val Tyr Tyr Arg Asp Ser Arg Asn Pro Leu Trp Lys Gly Pro Ala Lys Leu Leu Trp Lys Gly Glu Gly Ala Val Val Ile Gln Asp Asn Ser Asp Ile Lys Val Val Pro Arg Arg Lys Ala Lys Ile Ile Arg Asp Tyr Gly Lys Gln Met Ala Gly Asp Asp Cys Val Ala Ser Arg Gln Asp Glu Asp (SEQ ID NO:4).

As noted above, it will be understood that any combination of the mutations disclosed above may be suitable and therefore be utilized as an IA-pol-based adenoviral HIV vaccine of the present invention, either when administered alone or in a combined modality regime and/or a prime-boost regimen. For example, it may be possible to mutate only 2 of the 3 residues within the respective reverse transcriptase, RNase-H, and integrase coding regions while still abolishing these enzymatic activities. However, the IA-pol construct described above and disclosed as SEQ ID NO:3, as well as the expressed protein (SEQ ID NO:4;) is preferred. It is also preferred that at least one mutation be present in each of the three catalytic domains.

Another aspect of this portion of the invention are codon optimized HIV-1 Pol-based vaccine constructions which comprise a eukaryotic trafficking signal peptide such as from tPA (tissue-type plasminogen activator) or by a leader peptide such as is found in highly expressed mammalian proteins such as immunoglobulin leader peptides. Any functional leader peptide may be tested for efficacy. However, a preferred embodiment of the present invention, as with HIV-1 Nef constructs shown herein, is to provide for a HIV-1 Pol mutant adenoviral vaccine construction wherein the pol coding region or a portion thereof is operatively linked to a leader peptide, preferably a leader peptide from human tPA. In other words, a codon optimized HIV-1 Pol mutant such as IA-Pol (SEQ ID NO:4) may also comprise a leader peptide at the amino terminal portion of the protein, which may effect cellular trafficking and hence, immunogenicity of the expressed protein within the host cell. As noted in Figure 16A-B, a DNA vector which may be utilized to practice the present invention may be modified by known recombinant DNA methodology to contain a leader signal

peptide of interest, such that downstream cloning of the modified HIV-1 protein of interest results in a nucleotide sequence which encodes a modified HIV-1 tPA/Pol protein. In the alternative, as noted above, insertion of a nucleotide sequence which encodes a leader peptide may be inserted into a DNA vector housing the open reading frame for the Pol protein of interest. Regardless of the cloning strategy, the end result is a polynucleotide vaccine which comprises vector components for effective gene expression in conjunction with nucleotide sequences which encode a modified HIV-1 Pol protein of interest, including but not limited to a HIV-1 Pol protein which contains a leader peptide. The amino acid sequence of the human tPA leader utilized herein is as follows: MDAMKRGLCCVLLLCGAVFVSPSEISS (SEQ ID NO:17). Therefore, another aspect of the present invention is to generate HIV-1 Pol-based vaccine constructions which comprise a eukaryotic trafficking signal peptide such as from tPA. To this end, the present invention relates to a DNA molecule which encodes a codon optimized wt-pol DNA construct wherein the protease (PR) activity is deleted and a human tPA leader sequence is fused to the 5' end of the coding region. A DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:5, the open reading frame disclosed herein as SEQ ID NO:6.

5

10

15

20

25

30

35

To this end, the present invention relates to a DNA molecule which encodes a codon optimized wt-pol DNA construct wherein the protease (PR) activity is deleted and a human tPA leader sequence is fused to the 5' end of the coding region (herein, "tPA-wt-pol"). A DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:5, the open reading frame being contained from an initiating Met residue at nucleotides 8-10 to a termination codon from nucleotides 2633-2635. SEQ ID NO:5 is as follows:

GATCACCATG GATGCAATGA AGAGAGGGCT CTGCTGTGTG CTGCTGTGT GTGGAGCAGT
CTTCGTTTCG CCCAGCGAGA TCTCCGCCC CATCTCCCC ATTGAGACTG TGCCTGTGAA
GCTGAAGCCT GGCATGGATG GCCCCAAGGT GAAGCAGTGG CCCCTGACTG AGGAGAAGAT
CAAGGCCCTG GTGGAAATCT GCACTGAGAT GGAGAAGGAG GGCAAAATCT CCAAGATTGG
CCCCGAGAAC CCCTACAACA CCCCTGTGTT TGCCCATCAAG AAGAAGGACT CCAACAAGTG
GAGGAAGCTG GTGGACTTCA GGGAGCTGAA CAAGAGGACC CAGGACTTCT GGGAGGTGCA
GCTGGGCATC CCCCACCCCG CTGGCCTGAA GAAGAAGAAG TCTGTGACTG TGCTGGATGT
GGGGGATGCC TACTTCTCTG TGCCCCTGGA TGAGGACTTC AGGAAGTACA CTGCTCTAC
CATCCCCTCC ATCAACAATG AGACCCCTGG CATCAGGTAC AGGAAGTACA TGCTGCCCCA
GGGCTGGAAG GGCTCCCCTG CCATCTTCCA GTCCTCCATG ACCAAGATCC TGGAGCCCTT
CAGGAAGCAG AACCCTGACA TTGTGATCTA CCAGTACATG GATGACCTGT ATGTGGGCTC
TGACCTGGAG ATTGGGCAGC ACAGGACCAA GATTGAGGAG CTGAGGCAGC ACCTGCTGAG

GTGGGGCCTG ACCACCCCTG ACAAGAAGCA CCAGAAGGAG CCCCCCTTCC TGTGGATGGG CTATGAGCTG CACCCCGACA AGTGGACTGT GCAGCCCATT GTGCTGCCTG AGAAGGACTC CTGGACTGTG AATGACATCC AGAAGCTGGT GGGCAAGCTG AACTGGGCCT CCCAAATCTA CCCTGGCATC AAGGTGAGGC AGCTGTGCAA GCTGCTGAGG GGCACCAAGG CCCTGACTGA GGTGATCCCC CTGACTGAGG AGGCTGAGCT GGAGCTGGCT GAGAACAGGG AGATCCTGAA 5 GGAGCCTGTG CATGGGGTGT ACTATGACCC CTCCAAGGAC CTGATTGCTG AGATCCAGAA GCAGGGCCAG GGCCAGTGGA CCTACCAAAT CTACCAGGAG CCCTTCAAGA ACCTGAAGAC TGGCAAGTAT GCCAGGATGA GGGGGGCCCA CACCAATGAT GTGAAGCAGC TGACTGAGGC TGTGCAGAAG ATCACCACTG AGTCCATTGT GATCTGGGGC AAGACCCCCA AGTTCAAGCT 10 GCCCATCCAG AAGGAGACCT GGGAGACCTG GTGGACTGAG TACTGGCAGG CCACCTGGAT CCCTGAGTGG GAGTTTGTGA ACACCCCCC CCTGGTGAAG CTGTGGTACC AGCTGGAGAA GGAGCCCATT GTGGGGGCTG AGACCTTCTA TGTGGATGGG GCTGCCAACA GGGAGACCAA GCTGGCCAAG GCTGGCTATG TGACCAACAG GGGCAGGCAG AAGGTGGTGA CCCTGACTGA CACCACCAAC CAGAAGACTG AGCTCCAGGC CATCTACCTG GCCCTCCAGG ACTCTGGCCT 15 GGAGGTGAAC ATTGTGACTG ACTCCCAGTA TGCCCTGGGC ATCATCCAGG CCCAGCCTGA TCAGTCTGAG TCTGAGCTGG TGAACCAGAT CATTGAGCAG CTGATCAAGA AGGAGAAGGT GTACCTGGCC TGGGTGCCTG CCCACAAGGG CATTGGGGGC AATGAGCAGG TGGACAAGCT GGTGTCTGCT GGCATCAGGA AGGTGCTGTT CCTGGATGGC ATTGACAAGG CCCAGGATGA GCATGAGAAG TACCACTCCA ACTGGAGGGC TATGGCCTCT GACTTCAACC TGCCCCCTGT 20 GGTGGCTAAG GAGATTGTGG CCTCCTGTGA CAAGTGCCAG CTGAAGGGGG AGGCCATGCA TGGGCAGGTG GACTGCTCCC CTGGCATCTG GCAGCTGGAC TGCACCCACC TGGAGGGCAA GGTGATCCTG GTGGCTGTGC ATGTGGCCTC CGGCTACATT GAGGCTGAGG TGATCCCTGC TGAGACAGGC CAGGAGACTG CCTACTTCCT GCTGAAGCTG GCTGGCAGGT GGCCTGTGAA GACCATCCAC ACTGACAATG GCTCCAACTT CACTGGGGCC ACAGTGAGGG CTGCCTGCTG 25 GTGGGCTGGC ATCAAGCAGG AGTTTGGCAT CCCCTACAAC CCCCAGTCCC AGGGGGTGGT GGAGTCCATG AACAAGGAGC TGAAGAAGAT CATTGGGCAG GTGAGGGACC AGGCTGAGCA CCTGAAGACA GCTGTGCAGA TGGCTGTGTT CATCCACAAC TTCAAGAGGA AGGGGGGCAT CGGGGGCTAC TCCGCTGGGG AGAGGATTGT GGACATCATT GCCACAGACA TCCAGACCAA GGAGCTCCAG AAGCAGATCA CCAAGATCCA GAACTTCAGG GTGTACTACA GGGACTCCAG 30 GAACCCCTG TGGAAGGGCC CTGCCAAGCT GCTGTGGAAG GGGGAGGGGG CTGTGGTGAT CCAGGACAAC TCTGACATCA AGGTGGTGCC CAGGAGGAAG GCCAAGATCA TCAGGGACTA TGGCAAGCAG ATGGCTGGGG ATGACTGTGT GGCCTCCAGG CAGGATGAGG ACTAAAGCCC GGGCAGATCT (SEO ID NO:5).

The open reading frame of the wild type tPA-pol construct disclosed as SEQ ID NO:5 contains 875 amino acids, disclosed herein as SEQ ID NO:6, as follows:

Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Cys Gly

35

Ala Val Phe Val Ser Pro Ser Glu Ile Ser Ala Pro Ile Ser Pro Ile Glu Thr Val Pro Val Lys Leu Lys Pro Gly Met Asp Gly Pro Lys Val Lys Gln Trp Pro Leu Thr Glu Glu Lys Ile Lys Ala Leu Val Glu Ile Cys Thr Glu Met Glu Lys Glu Gly Lys Ile Ser Lys Ile Gly Pro Glu Asn Pro Tyr Asn Thr Pro Val Phe Ala Ile Lys Lys Lys Asp Ser Thr Lys Trp Arg Lys Leu Val Asp Phe Arg Glu Leu Asn Lys Arg Thr Gln Asp Phe Trp Glu Val Gln Leu Gly Ile Pro His Pro Ala Gly Leu Lys Lys Lys Lys Ser Val Thr Val Leu Asp Val Gly Asp Ala Tyr Phe Ser Val Pro Leu Asp Glu Asp Phe Arg Lys Tyr Thr Ala Phe Thr Ile Pro Ser Ile Asn Asn Glu Thr Pro Gly Ile Arg Tyr Gln Tyr Asn Val Leu 10 Pro Gln Gly Trp Lys Gly Ser Pro Ala Ile Phe Gln Ser Ser Met Thr Lys Ile Leu Glu Pro Phe Arg Lys Gln Asn Pro Asp Ile Val Ile Tyr Gln Tyr Met Asp Asp Leu Tyr Val Gly Ser Asp Leu Glu Ile Gly Gln His Arg Thr Lys Ile Glu Glu Leu Arg Gln His Leu Leu Arg Trp Gly Leu Thr Thr Pro Asp Lys Lys His Gln Lys Glu Pro Pro Phe Leu Trp 15 Met Gly Tyr Glu Leu His Pro Asp Lys Trp Thr Val Gln Pro Ile Val Leu Pro Glu Lys Asp Ser Trp Thr Val Asn Asp Ile Gln Lys Leu Val Gly Lys Leu Asn Trp Ala Ser Gln Ile Tyr Pro Gly Ile Lys Val Arg Gln Leu Cys Lys Leu Leu Arg Gly Thr Lys Ala Leu Thr Glu Val Ile Pro Leu Thr Glu Glu Ala Glu Leu Glu Leu Ala Glu Asn Arg Glu Ile 20 Leu Lys Glu Pro Val His Gly Val Tyr Tyr Asp Pro Ser Lys Asp Leu Ile Ala Glu Ile Gln Lys Gln Gly Gln Gly Gln Trp Thr Tyr Gln Ile Tyr Gln Glu Pro Phe Lys Asn Leu Lys Thr Gly Lys Tyr Ala Arg Met Arg Gly Ala His Thr Asn Asp Val Lys Gln Leu Thr Glu Ala Val Gln Lys Ile Thr Thr Glu Ser Ile Val Ile Trp Gly Lys Thr Pro Lys Phe 25 Lys Leu Pro Ile Gln Lys Glu Thr Trp Glu Thr Trp Trp Thr Glu Tyr Trp Gln Ala Thr Trp Ile Pro Glu Trp Glu Phe Val Asn Thr Pro Pro Leu Val Lys Leu Trp Tyr Gln Leu Glu Lys Glu Pro Ile Val Gly Ala Glu Thr Phe Tyr Val Asp Gly Ala Ala Asn Arg Glu Thr Lys Leu Gly Lys Ala Gly Tyr Val Thr Asn Arg Gly Arg Gln Lys Val Val Thr Leu 30 Thr Asp Thr Thr Asn Gln Lys Thr.Glu.Leu Gln Ala Ile Tyr Leu Ala Leu Gln Asp Ser Gly Leu Glu Val Asn Ile Val Thr Asp Ser Gln Tyr Ala Leu Gly Ile Ile Gln Ala Gln Pro Asp Gln Ser Glu Ser Glu Leu Val Asn Gln Ile Ile Glu Gln Leu Ile Lys Lys Glu Lys Val Tyr Leu Ala Trp Val Pro Ala His Lys Gly Ile Gly Gly Asn Glu Gln Val Asp 35 Lys Leu Val Ser Ala Gly Ile Arg Lys Val Leu Phe Leu Asp Gly Ile

Asp Lys Ala Gln Asp Glu His Glu Lys Tyr His Ser Asn Trp Arg Ala Met Ala Ser Asp Phe Asn Leu Pro Pro Val Val Ala Lys Glu Ile Val Ala Ser Cys Asp Lys Cys Gln Leu Lys Gly Glu Ala Met His Gly Gln Val Asp Cys Ser Pro Gly Ile Trp Gln Leu Asp Cys Thr His Leu Glu Gly Lys Val Ile Leu Val Ala Val His Val Ala Ser Gly Tyr Ile Glu Ala Glu Val Ile Pro Ala Glu Thr Gly Gln Glu Thr Ala Tyr Phe Leu Leu Lys Leu Ala Gly Arg Trp Pro Val Lys Thr Ile His Thr Asp Asn Gly Ser Asn Phe Thr Gly Ala Thr Val Arg Ala Ala Cys Trp Trp Ala Gly Ile Lys Gln Glu Phe Gly Ile Pro Tyr Asn Pro Gln Ser Gln Gly Val Val Glu Ser Met Asn Lys Glu Leu Lys Lys Ile Ile Gly Gln Val Arg Asp Gln Ala Glu His Leu Lys Thr Ala Val Gln Met Ala Val Phe Ile His Asn Phe Lys Arg Lys Gly Gly Ile Gly Gly Tyr Ser Ala Gly Glu Arg Ile Val Asp Ile Ile Ala Thr Asp Ile Gln Thr Lys Glu Leu Gln Lys Gln Ile Thr Lys Ile Gln Asn Phe Arg Val Tyr Tyr Arg Asp Ser Arg Asn Pro Leu Trp Lys Gly Pro Ala Lys Leu Leu Trp Lys Gly Glu Gly Ala Val Val Ile Gln Asp Asn Ser Asp Ile Lys Val Val Pro Arg Arg Lys Ala Lys Ile Ile Arg Asp Tyr Gly Lys Gln Met Ala Gly Asp Asp Cys Val Ala Ser Arg Gln Asp Glu Asp (SEQ ID NO:6).

10

15

20

25

30

35

The present invention also relates to a codon optimized HIV-1 Pol mutant contained within a recombinant adenoviral vector such as IA-Pol (SEQ ID NO:4) which comprises a leader peptide at the amino terminal portion of the protein, which may effect cellular trafficking and hence, immunogenicity of the expressed protein within the host cell. Any such adenoviral-based HIV-1 DNA pol mutant disclosed in the above paragraphs is suitable for fusion downstream of a leader peptide, such as a leader peptide including but not limited to the human tPA leader sequence. Therefore, any such leader peptide-based HIV-1 pol mutant construct may include but is not limited to a mutated DNA molecule which effectively alters the catalytic activity of the RT, RNase and/or IN region of the expressed protein, resulting in at least substantially decreased enzymatic activity one or more of the RT, RNase H and/or IN functions of HIV-1 Pol. In a preferred embodiment of this portion of the invention, a leader peptide/HIV-1 DNA pol construct contains a mutation or mutations within the Pol coding region which effectively abolishes RT, RNase H and IN activity. An especially preferable HIV-1 DNA pol construct is a DNA molecule which contains at least one point mutation which alters the active site and catalytic activity within the RT, RNase H and IN domains of Pol, such that each activity is at least substantially abolished, and preferably totally abolished. Such a HIV-1 Pol mutant will most likely

5

10

15

20

25

30

35

comprise at least one point mutation in or around each catalytic domain responsible for RT, RNase H and IN activity, respectfully. An especially preferred embodiment of this portion of the invention relates to a human tPA leader fused to the IA-Pol protein comprising the nine mutations shown in Table 1. The DNA molecule is disclosed herein as SEQ ID NO:7 and the expressed tPA-IA Pol protein comprises a fusion junction as shown in Figure 18. The complete amino acid sequence of the expressed protein is set forth in SEQ ID NO:8. To this end, SEQ ID NO:7 discloses the nucleotide sequence which codes for a human tPA leader fused to the IA Pol protein comprising the nine mutations shown in Table 1 (herein, "tPA-opt-IApol"). The open reading frame begins with the initiating Met (nucleotides 8-10) and terminates with a "TAA" codon at nucleotides 2633-2635. The nucleotide sequence encoding tPA-IAPol is also disclosed as follows: GATCACCATG GATGCAATGA AGAGAGGGCT CTGCTGTGTG CTGCTGCTGT GTGGAGCAGT CTTCGTTTCG CCCAGCGAGA TCTCCGCCCC CATCTCCCCC ATTGAGACTG TGCCTGTGAA GCTGAAGCCT GGCATGGATG GCCCCAAGGT GAAGCAGTGG CCCCTGACTG AGGAGAAGAT CAAGGCCCTG GTGGAAATCT GCACTGAGAT GGAGAAGGAG GGCAAAATCT CCAAGATTGG CCCCGAGAAC CCCTACAACA CCCCTGTGTT TGCCATCAAG AAGAAGGACT CCACCAAGTG GAGGAAGCTG GTGGACTTCA GGGAGCTGAA CAAGAGGACC CAGGACTTCT GGGAGGTGCA GCTGGGCATC CCCCACCCG CTGGCCTGAA GAAGAAGAAG TCTGTGACTG TGCTGGCTGT GGGGGATGCC TACTTCTCTG TGCCCCTGGA TGAGGACTTC AGGAAGTACA CTGCCTTCAC CATCCCCTCC ATCAACAATG AGACCCCTGG CATCAGGTAC CAGTACAATG TGCTGCCCCA GGGCTGGAAG GGCTCCCCTG CCATCTTCCA GTCCTCCATG ACCAAGATCC TGGAGCCCTT CAGGAAGCAG AACCCTGACA TTGTGATCTA CCAGTACATG GCTGCCCTGT ATGTGGGCTC TGACCTGGAG ATTGGGCAGC ACAGGACCAA GATTGAGGAG CTGAGGCAGC ACCTGCTGAG GTGGGGCCTG ACACCCCTG ACAGAAGCA CCAGAAGGAG CCCCCCTTCC TGTGGATGGG CTATGAGCTG CACCCCGACA AGTGGACTGT GCAGCCCATT GTGCTGCCTG AGAAGGACTC CTGGACTGTG AATGACATCC AGAAGCTGGT GGGCAAGCTG AACTGGGCCT CCCAAATCTA CCCTGGCATC AAGGTGAGGC AGCTGTGCAA GCTGCTGAGG GGCACCAAGG CCCTGACTGA GGTGATCCCC CTGACTGAGG AGGCTGAGCT GGAGCTGGCT GAGAACAGGG AGATCCTGAA GGAGCCTGTG CATGGGGTGT ACTATGACCC CTCCAAGGAC CTGATTGCTG AGATCCAGAA GCAGGGCCAG GGCCAGTGGA CCTACCAAAT CTACCAGGAG CCCTTCAAGA ACCTGAAGAC TGGCAAGTAT GCCAGGATGA GGGGGGCCCA CACCAATGAT GTGAAGCAGC TGACTGAGGC TGTGCAGAAG ATCACCACTG AGTCCATTGT GATCTGGGGC AAGACCCCCA AGTTCAAGCT GCCCATCCAG AAGGAGACCT GGGAGACCTG GTGGACTGAG TACTGGCAGG CCACCTGGAT CCCTGAGTGG GAGTTTGTGA ACACCCCCC CCTGGTGAAG CTGTGGTACC AGCTGGAGAA GGAGCCCATT GTGGGGGCTG AGACCTTCTA TGTGGCTGGG GCTGCCAACA GGGAGACCAA

GCTGGGCAAG GCTGGCTATG TGACCAACAG GGGCAGGCAG AAGGTGGTGA CCCTGACTGA CACCACCAAC CAGAAGACTG CCCTCCAGGC CATCTACCTG GCCCTCCAGG ACTCTGGCCT GGAGGTGAAC ATTGTGACTG CCTCCCAGTA TGCCCTGGGC ATCATCCAGG CCCAGCCTGA TCAGTCTGAG TCTGAGCTGG TGAACCAGAT CATTGAGCAG CTGATCAAGA AGGAGAAGGT GTACCTGGCC TGGGTGCCTG CCCACAAGGG CATTGGGGGC AATGAGCAGG TGGACAAGCT GGTGTCTGCT GGCATCAGGA AGGTGCTGTT CCTGGATGGC ATTGACAAGG CCCAGGATGA GCATGAGAAG TACCACTCCA ACTGGAGGGC TATGGCCTCT GACTTCAACC TGCCCCCTGT GGTGGCTAAG GAGATTGTGG CCTCCTGTGA CAAGTGCCAG CTGAAGGGGG AGGCCATGCA TGGGCAGGTG GACTGCTCCC CTGGCATCTG GCAGCTGGCC TGCACCCACC TGGAGGGCAA 10 GGTGATCCTG GTGGCTGTGC ATGTGGCCTC CGGCTACATT GAGGCTGAGG TGATCCCTGC TGAGACAGGC CAGGAGACTG CCTACTTCCT GCTGAAGCTG GCTGGCAGGT GGCCTGTGAA GACCATCCAC ACTGCCAATG GCTCCAACTT CACTGGGGCC ACAGTGAGGG CTGCCTGCTG GTGGGCTGGC ATCAAGCAGG AGTTTGGCAT CCCCTACAAC CCCCAGTCCC AGGGGGTGGT GGCCTCCATG AACAAGGAGC TGAAGAAGAT CATTGGGCAG GTGAGGGACC AGGCTGAGCA 15 CCTGAAGACA GCTGTGCAGA TGGCTGTGTT CATCCACAAC TTCAAGAGGA AGGGGGGCAT CGGGGGCTAC TCCGCTGGGG AGAGGATTGT GGACATCATT GCCACAGACA TCCAGACCAA GGAGCTCCAG AAGCAGATCA CCAAGATCCA GAACTTCAGG GTGTACTACA GGGACTCCAG GAACCCCTG TGGAAGGGCC CTGCCAAGCT GCTGTGGAAG GGGGAGGGGG CTGTGGTGAT CCAGGACAAC TCTGACATCA AGGTGGTGCC CAGGAGGAAG GCCAAGATCA TCAGGGACTA 20 TGGCAAGCAG ATGGCTGGGG ATGACTGTGT GGCCTCCAGG CAGGATGAGG ACTAAAGCCC GGGCAGATCT (SEQ ID NO:7).

The open reading frame of the tPA-IA-pol construct disclosed as SEQ ID NO:7 contains 875 amino acids, disclosed herein as tPA-IA-Pol and SEQ ID NO:8, as follows:

25 Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly Ala Val Phe Val Ser Pro Ser Glu Ile Ser Ala Pro Ile Ser Pro Ile Glu Thr Val Pro Val Lys Leu Lys Pro Gly Met Asp Gly Pro Lys Val Lys Gln Trp Pro Leu Thr Glu Glu Lys Ile Lys Ala Leu Val Glu Ile Cys Thr Glu Met Glu Lys Glu Gly Lys Ile Ser Lys Ile Gly Pro Glu 30 Asn Pro Tyr Asn Thr Pro Val Phe Ala Ile Lys Lys Lys Asp Ser Thr Lys Trp Arg Lys Leu Val Asp Phe Arg Glu Leu Asn Lys Arg Thr Gln Asp Phe Trp Glu Val Gln Leu Gly Ile Pro His Pro Ala Gly Leu Lys Lys Lys Lys Ser Val Thr Val Leu Ala Val Gly Asp Ala Tyr Phe Ser Val Pro Leu Asp Glu Asp Phe Arg Lys Tyr Thr Ala Phe Thr Ile Pro Ser Ile Asn Asn Glu Thr Pro Gly Ile Arg Tyr Gln Tyr Asn Val Leu Pro Gln Gly Trp Lys Gly Ser Pro Ala Ile Phe Gln Ser Ser Met Thr

Lys Ile Leu Glu Pro Phe Arg Lys Gln Asn Pro Asp Ile Val Ile Tyr Gln Tyr Met Ala Ala Leu Tyr Val Gly Ser Asp Leu Glu Ile Gly Gln His Arg Thr Lys Ile Glu Glu Leu Arg Gln His Leu Leu Arg Trp Gly Leu Thr Thr Pro Asp Lys Lys His Gln Lys Glu Pro Pro Phe Leu Trp Met Gly Tyr Glu Leu His Pro Asp Lys Trp Thr Val Gln Pro Ile Val Leu Pro Glu Lys Asp Ser Trp Thr Val Asn Asp Ile Gln Lys Leu Val Gly Lys Leu Asn Trp Ala Ser Gln Ile Tyr Pro Gly Ile Lys Val Arg Gln Leu Cys Lys Leu Leu Arg Gly Thr Lys Ala Leu Thr Glu Val Ile Pro Leu Thr Glu Glu Ala Glu Leu Glu Leu Ala Glu Asn Arg Glu Ile Leu Lys Glu Pro Val His Gly Val Tyr Tyr Asp Pro Ser Lys Asp Leu 10 Ile Ala Glu Ile Gln Lys Gln Gly Gln Gly Gln Trp Thr Tyr Gln Ile Tyr Gln Glu Pro Phe Lys Asn Leu Lys Thr Gly Lys Tyr Ala Arg Met Arg Gly Ala His Thr Asn Asp Val Lys Gln Leu Thr Glu Ala Val Gln Lys Ile Thr Thr Glu Ser Ile Val Ile Trp Gly Lys Thr Pro Lys Phe Lys Leu Pro Ile Gln Lys Glu Thr Trp Glu Thr Trp Trp Thr Glu Tyr 15 Trp Gln Ala Thr Trp Ile Pro Glu Trp Glu Phe Val Asn Thr Pro Pro Leu Val Lys Leu Trp Tyr Gln Leu Glu Lys Glu Pro Ile Val Gly Ala Glu Thr Phe Tyr Val Ala Gly Ala Ala Asn Arg Glu Thr Lys Leu Gly Lys Ala Gly Tyr Val Thr Asn Arg Gly Arg Gln Lys Val Val Thr Leu 20 Thr Asp Thr Thr Asn Gln Lys Thr Ala Leu Gln Ala Ile Tyr Leu Ala Leu Gln Asp Ser Gly Leu Glu Val Asn Ile Val Thr Ala Ser Gln Tyr Ala Leu Gly Ile Ile Gln Ala Gln Pro Asp Gln Ser Glu Ser Glu Leu Val Asn Gln Ile Ile Glu Gln Leu Ile Lys Lys Glu Lys Val Tyr Leu Ala Trp Val Pro Ala His Lys Gly Ile Gly Gly Asn Glu Gln Val Asp Lys Leu Val Ser Ala Gly Ile Arg Lys Val Leu Phe Leu Asp Gly Ile 25 Asp Lys Ala Gln Asp Glu His Glu Lys Tyr His Ser Asn Trp Arg Ala Met Ala Ser Asp Phe Asn Leu Pro Pro Val Val Ala Lys Glu Ile Val Ala Ser Cys Asp Lys Cys Gln Leu Lys Gly Glu Ala Met His Gly Gln Val Asp Cys Ser Pro Gly Ile Trp Gln Leu Ala Cys Thr His Leu Glu Gly Lys Val Ile Leu Val Ala Val His Val Ala Ser Gly Tyr Ile Glu 30 Ala Glu Val Ile Pro Ala Glu Thr Gly Gln Glu Thr Ala Tyr Phe Leu Leu Lys Leu Ala Gly Arg Trp Pro Val Lys Thr Ile His Thr Ala Asn Gly Ser Asn Phe Thr Gly Ala Thr Val Arg Ala Ala Cys Trp Trp Ala Gly Ile Lys Gln Glu Phe Gly Ile Pro Tyr Asn Pro Gln Ser Gln Gly Val Val Ala Ser Met Asn Lys Glu Leu Lys Lys Ile Ile Gly Gln Val 35 Arg Asp Gln Ala Glu His Leu Lys Thr Ala Val Gln Met Ala Val Phe

Ile His Asn Phe Lys Arg Lys Gly Gly Ile Gly Gly Tyr Ser Ala Gly Glu Arg Ile Val Asp Ile Ile Ala Thr Asp Ile Gln Thr Lys Glu Leu Gln Lys Gln Ile Thr Lys Ile Gln Asn Phe Arg Val Tyr Tyr Arg Asp Ser Arg Asn Pro Leu Trp Lys Gly Pro Ala Lys Leu Leu Trp Lys Gly Glu Gly Ala Val Val Ile Gln Asp Asn Ser Asp Ile Lys Val Val Pro Arg Arg Lys Ala Lys Ile Ile Arg Asp Tyr Gly Lys Gln Met Ala Gly Asp Asp Cys Val Ala Ser Arg Gln Asp Glu Asp (SEQ ID NO:8).

EXAMPLE 18

CODON OPTIMIZED HIV-1 NEF AND CODON OPTIMIZED HIV-1 NEF MODIFICATIONS

10

15

20

25

30

35

Codon optimized version of HIV-1 Nef and HIV-1 Nef modifications are essentially as described in U.S. Application Serial No. 09/738,782, filed December 15, 2000 and PCT International Application PCT/US00/34162, also filed December 15, 2000, both documents which are hereby incorporated by reference. As disclosed within the above-mentioned documents, particular embodiments of codon optimized Nef and Nef modifications relate to a DNA molecule encoding HIV-1 Nef from the HIV-1 ifrl isolate wherein the codons are optimized for expression in a mammalian system such as a human. The DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:9, while the expressed open reading frame is disclosed herein as SEQ ID NO:10. Another embodiment of Nef-based coding regions for use in the adenoviral vectors of the present invention comprise a codon optimized DNA molecule encoding a protein containing the human plasminogen activator (tpa) leader peptide fused with the NH2-terminus of the HIV-1 Nef polypeptide. The DNA molecule which encodes this protein is disclosed herein as SEO ID NO:11, while the expressed open reading frame is disclosed herein as SEO ID NO:12. Another modified Nef optimized coding region relates to a DNA molecule encoding optimized HIV-1 Nef wherein the open reading frame codes for modifications at the amino terminal myristylation site (Gly-2 to Ala-2) and substitution of the Leu-174-Leu-175 dileucine motif to Ala-174-Ala-175, herein described as opt nef (G2A, LLAA). The DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:13, while the expressed open reading frame is disclosed herein as SEQ ID NO:14. An additional embodiment relates to a DNA molecule encoding optimized HIV-1 Nef wherein the amino terminal myristylation site and dileucine motif have been deleted, as well as comprising a tPA leader peptide. This DNA molecule, opt tpanef (LLAA), comprises an open reading frame which

encodes a Nef protein containing a tPA leader sequence fused to amino acid residue 6-216 of HIV-1 Nef (jfrl), wherein Leu-174 and Leu-175 are substituted with Ala-174 and Ala-175, herein referred to as opt tpanef (LLAA) is disclosed herein as SEQ ID NO:15, while the expressed open reading frame is disclosed herein as SEQ ID NO:16.

As disclosed in the above-identified documents (U.S. Application Serial No. 09/738,782 and PCT International Application PCT/US00/34162) and reiterated herein, the following nef-based nucleotide and amino acid sequences which comprise the respective open reading frame are as follows:

5

10

15

20

25

30

35

1. The nucleotide sequence of the codon optimized version of HIV-1 jrfl nef gene is disclosed herein as SEQ ID NO:9, as shown herein:

GATCTGCCAC CATGGGCGGC AAGTGGTCCA AGAGGTCCGT GCCCGGCTGG TCCACCGTGA
GGGAGAGGAT GAGGAGGGCC GAGCCCGCCG CCGACAGGGT GAGGAGGACC GAGCCCGCCG
CCGTGGGCGT GGGCGCCGTG TCCAGGGACC TGGAGAAGCA CGGCGCCATC ACCTCCTCA
ACACCGCCGC CACCAACGCC GACTGCGCCT GGCTGGAGGC CCAGGAGGAC GAGGAGGTGG
GCTTCCCCGT GAGGCCCAG GTGCCCCTGA GGCCCATGAC CTACAAGGGC GCCGTGGACC
TGTCCCACTT CCTGAAGGAG AAGGGCGGCC TGGAGGGCCT GATCCACTCC CAGAAGAGGC
AGGACATCCT GGACCTGTGG GTGTACCACA CCCAGGGCTA CTTCCCCGAC TGGCAGAACT
ACACCCCCGG CCCCGGCATC AGGTTCCCCC TGACCTTCGG CTGGTGCTTC AAGCTGGTGC
CCGTGGAGCC CGAGAAGGTG GAGGAGGCCA ACGAGGGCGA GAACAACTGC CTGCTGCACC
CCATGTCCCA GCACGGCATC GAGGACCCCG AGAAGGAGGT GCTGGAGTGG AGGTTCGACT
CCAAGCTGGC CTTCCACCAC GTGGCCAGGG AGCTGCACCC CGAGTACTAC AAGGACTGCT
AAAGCCCGGG C (SEQ ID NO:9).

Preferred codon usage is as follows: Met (ATG), Gly (GGC), Lys (AAG), Trp (TGG), Ser (TCC), Arg (AGG), Val (GTG), Pro (CCC), Thr (ACC), Glu (GAG); Leu (CTG), His (CAC), Ile (ATC), Asn (AAC), Cys (TGC), Ala (GCC), Gln (CAG), Phe (TTC) and Tyr (TAC). For an additional discussion relating to mammalian (human) codon optimization, see WO 97/31115 (PCT/US97/02294), which is hereby incorporated by reference. See also Figure 19A-B for a comparion of wild type vs. codon optimized nucleotides comprising the open reading frame of HIV-Nef.

The open reading frame for SEQ ID NO:9 above comprises an initiating methionine residue at nucleotides 12-14 and a "TAA" stop codon from nucleotides 660-662. The open reading frame of SEQ ID NO:9 provides for a 216 amino acid HIV-1 Nef protein expressed through utilization of a codon optimized DNA vaccine vector. The 216 amino acid HIV-1 Nef (jfrl) protein is disclosed herein as SEQ ID NO:10, and as follows:

Met Gly Gly Lys Trp Ser Lys Arg Ser Val Pro Gly Trp Ser Thr Val

Arg Glu Arg Met Arg Arg Ala Glu Pro Ala Ala Asp Arg Val Arg Arg Thr Glu Pro Ala Ala Val Gly Val Gly Ala Val Ser Arg Asp Leu Glu Lys His Gly Ala Ile Thr Ser Ser Asn Thr Ala Ala Thr Asn Ala Asp Cys Ala Trp Leu Glu Ala Gln Glu Asp Glu Glu Val Gly Phe Pro Val Arg Pro Gln Val Pro Leu Arg Pro Met Thr Tyr Lys Gly Ala Val Asp Leu Ser His Phe Leu Lys Glu Lys Gly Gly Leu Glu Gly Leu Ile His Ser Gln Lys Arg Gln Asp Ile Leu Asp Leu Trp Val Tyr His Thr Gln Gly Tyr Phe Pro Asp Trp Gln Asn Tyr Thr Pro Gly Pro Gly Ile Arg Phe Pro Leu Thr Phe Gly Trp Cys Phe Lys Leu Val Pro Val Glu Pro Glu Lys Val Glu Glu Ala Asn Glu Gly Glu Asn Asn Cys Leu His Pro Met Ser Gln His Gly Ile Glu Asp Pro Glu Lys Glu Val Leu Glu His Pro Arg Phe Asp Ser Lys Leu Ala Phe His His Val Ala Arg Glu Leu His Pro Glu Tyr Tyr Lys Asp Cys (SEQ ID NO:10).

10

15

20

25

30

35

HIV-1 Nef is a 216 amino acid cytosolic protein which associates with the inner surface of the host cell plasma membrane through myristylation of Gly-2 (Franchini et al., 1986, Virology 155: 593-599). While not all possible Nef functions have been elucidated, it has become clear that correct trafficking of Nef to the inner plasma membrane promotes viral replication by altering the host intracellular environment to facilitate the early phase of the HIV-1 life cycle and by increasing the infectivity of progeny viral particles. In one aspect of the invention regarding codon-optimized, protein-modified polypeptides, the nef-encoding region of the adenovirus vector of the present invention is modified to contain a nucleotide sequence which encodes a heterologous leader peptide such that the amino terminal region of the expressed protein will contain the leader peptide. The diversity of function that typifies eukaryotic cells depends upon the structural differentiation of their membrane boundaries. To generate and maintain these structures, proteins must be transported from their site of synthesis in the endoplasmic reticulum to predetermined destinations throughout the cell. This requires that the trafficking proteins display sorting signals that are recognized by the molecular machinery responsible for route selection located at the access points to the main trafficking pathways. Sorting decisions for most proteins need to be made only once as they traverse their biosynthetic pathways since their final destination, the cellular location at which they perform their function, becomes their permanent residence. Maintenance of intracellular integrity depends in part on the selective sorting and accurate transport of proteins to their correct destinations. Defined sequence motifs exist in proteins which can act as 'address labels'. A number of sorting signals have

been found associated with the cytoplasmic domains of membrane proteins. An effective induction of CTL responses often required sustained, high level endogenous expression of an antigen. As membrane-association via myristylation is an essential requirement for most of Nef's function, mutants lacking myristylation, by glycine-to-alanine change, change of the dileucine motif and/or by substitution with a tpa leader sequence as described herein, will be functionally defective, and therefore will have improved safety profile compared to wild-type Nef for use as an HIV-1 vaccine component.

In another embodiment of this portion of the invention, either the DNA vector or the HIV-1 nef nucleotide sequence is modified to include the human tissue-specific plasminogen activator (tPA) leader. As shown in Figure 16A-B, a DNA vector may be modified by known recombinant DNA methodology to contain a leader signal peptide of interest, such that downstream cloning of the modified HIV-1 protein of interest results in a nucleotide sequence which encodes a modified HIV-1 tPA/Nef protein. In the alternative, as noted above, insertion of a nucleotide sequence which encodes a leader peptide may be inserted into a DNA vector housing the open reading frame for the Nef protein of interest. Regardless of the cloning strategy, the end result is a polynucleotide vaccine which comprises vector components for effective gene expression in conjunction with nucleotide sequences which encode a modified HIV-1 Nef protein of interest, including but not limited to a HIV-1 Nef protein which contains a leader peptide. The amino acid sequence of the human tPA leader utilized herein is as follows: MDAMKRGLCCVLLLCGAVFVSPSEISS (SEQ ID NO:17).

It has been shown that myristylation of Gly-2 in conjunction with a dileucine motif in the carboxy region of the protein is essential for Nef-induced down regulation of CD4 (Aiken et al., 1994, Cell 76: 853-864) via endocytosis. It has also been shown that Nef expression promotes down regulation of MHCI (Schwartz et al., 1996, Nature Medicine 2(3): 338-342) via endocytosis. The present invention relates in part to DNA vaccines which encode modified Nef proteins altered in trafficking and/or functional properties. The modifications introduced into the adenoviral vector HIV vaccines of the present invention include but are not limited to additions, deletions or substitutions to the nef open reading frame which results in the expression of a modified Nef protein which includes an amino terminal leader peptide, modification or deletion of the amino terminal myristylation site, and modification or deletion of the dileucine motif within the Nef protein and which alter function within the infected host cell. Therefore, a central theme of the DNA molecules and recombinant adenoviral HIV vaccines of the present invention is (1)

host administration and intracellular delivery of a codon optimized nef-based adenoviral HIV vaccine; (2) expression of a modified Nef protein which is immunogenic in terms of eliciting both CTL and Th responses; and, (3) inhibiting or at least altering known early viral functions of Nef which have been shown to promote HIV-1 replication and load within an infected host. Therefore, the nef coding region may be altered, resulting in a DNA vaccine which expresses a modified Nef protein wherein the amino terminal Gly-2 myristylation residue is either deleted or modified to express alternate amino acid residues. Also, the nef coding region may be altered so as to result in a DNA vaccine which expresses a modified Nef protein wherein the dileucine motif is either deleted or modified to express alternate amino acid residues. In addition, the adenoviral vector HIV vaccines of the present invention also relate to an isolated DNA molecule, regardless of codon usage, which expresses a wild type or modified Nef protein as described herein, including but not limited to modified Nef proteins which comprise a deletion or substitution of Gly 2, a deletion or substitution of Leu 174 and Leu 175 and/or inclusion of a leader sequence.

10

15

20

25

30

35

Therefore, specific Nef-based constructs further include the following, as exemplification's and not limitations. For example, the present invention relates to an adenoviral vector vaccine which encodes modified forms of HIV-1, an open reading frame which encodes a Nef protein which comprises a tPA leader sequence fused to amino acid residue 6-216 of HIV-1 Nef (jfrl) is referred to herein as opt tpanef. The nucleotide sequence comprising the open reading frame of opt tpanef is disclosed herein as SEQ ID NO:11, as shown below:

CATGGATGCA ATGAAGAGA GGCTCTGCTG TGTGCTGCTG CTGTGTGGAG CAGTCTTCGT
TTCGCCCAGC GAGATCTCCT CCAAGAGGTC CGTGCCCGGC TGGTCCACCG TGAGGGAGAG
GATGAGGAGG GCCGAGCCCG CCGCCGACAG GGTGAGGAGG ACCGAGCCCG CCGCCGTGGG
CGTGGGCGCC GTGTCCAGGG ACCTGGAGAA GCACGGCGCC ATCACCTCCT CCAACACCGC
CGCCACCAAC GCCGACTGCG CCTGGCTGGA GGCCCAGGAG GACGAGGAGG TGGGCTTCCC
CGTGAGGCCC CAGGTGCCCC TGAGGCCCAT GACCTACAAG GGCGCCGTGG ACCTGTCCCA
CTTCCTGAAG GAGAAGGGCG GCCTGGAGGG CCTGATCCAC TCCCAGAAGA GGCAGGACAT
CCTGGACCTG TGGGTGTACC ACACCCAGGG CTACTTCCCC GACTGGCAGA ACTACACCCC
CGGCCCCGGC ATCAGGTTCC CCCTGACCTT CGGCTGGTCC TTCAAGCTGG TGCCCGTGGA
GCCCGAGAAG GTGGAGGAG CCAACGAGGG CGAGAACAAC TGCCTGCTGC ACCCCATGTC
CCAGCACGGC ATCGAGGACC CCGAGAAGGA GGTGCTGGAG TGGAGGTTCG ACCCCATGTC
GGCCTTCCAC CACGTGGCCA GGGAGCTGCA CCCCGAGTAC TACAAGGACT GCTAAAGCC
(SEO ID NO:11).

The open reading frame for SEQ ID NO:11 comprises an initiating methionine

residue at nucleotides 2-4 and a "TAA" stop codon from nucleotides 713-715. The open reading frame of SEQ ID NO:3 provides for a 237 amino acid HIV-1 Nef protein which comprises a tPA leader sequence fused to amino acids 6-216 of HIV-1 Nef, including the dileucine motif at amino acid residues 174 and 175. This 237 amino acid tPA/Nef (jfrl) fusion protein is disclosed herein as SEQ ID NO:12, and is shown as follows:

Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Cys Gly Ala Val Phe Val Ser Pro Ser Glu Ile Ser Ser Lys Arg Ser Val Pro Gly Trp Ser Thr Val Arg Glu Arg Met Arg Arg Ala Glu Pro Ala Ala Asp Arg Val Arg Arg Thr Glu Pro Ala Ala Val Gly Val Gly Ala Val 10 Ser Arg Asp Leu Glu Lys His Gly Ala Ile Thr Ser Ser Asn Thr Ala Ala Thr Asn Ala Asp Cys Ala Trp Leu Glu Ala Gln Glu Asp Glu Glu Val Gly Phe Pro Val Arg Pro Gln Val Pro Leu Arg Pro Met Thr Tyr Lys Gly Ala Val Asp Leu Ser His Phe Leu Lys Glu Lys Gly Gly Leu Glu Gly Leu Ile His Ser Gln Lys Arg Gln Asp Ile Leu Asp Leu Trp 15 Val Tyr His Thr Gln Gly Tyr Phe Pro Asp Trp Gln Asn Tyr Thr Pro Gly Pro Gly Ile Arg Phe Pro Leu Thr Phe Gly Trp Cys Phe Lys Leu Val Pro Val Glu Pro Glu Lys Val Glu Glu Ala Asn Glu Gly Glu Asn Asn Cys Leu Leu His Pro Met Ser Gln His Gly Ile Glu Asp Pro Glu Lys Glu Val Leu Glu Trp Arg Phe Asp Ser Lys Leu Ala Phe His His 20 Val Ala Arg Glu Leu His Pro Glu Tyr Tyr Lys Asp Cys (SEQ ID NO:12). Therefore, this exemplified Nef protein, Opt tPA-Nef, contains both a tPA leader sequence as well as deleting the myristylation site of Gly-2A DNA molecule encoding HIV-1 Nef from the HIV-1 ifrl isolate wherein the codons are optimized for 25 expression in a mammalian system such as a human.

In another specific embodiment of the present invention, a DNA molecule is disclosed which encodes optimized HIV-1 Nef wherein the open reading frame of a recombinant adenoviral HIV vaccine encodes for modifications at the amino terminal myristylation site (Gly-2 to Ala-2) and substitution of the Leu-174-Leu-175 dileucine motif to Ala-174-Ala-175. This open reading frame is herein described as opt nef (G2A,LLAA) and is disclosed as SEQ ID NO:13, which comprises an initiating methionine residue at nucleotides 12-14 and a "TAA" stop codon from nucleotides 660-662. The nucleotide sequence of this codon optimized version of HIV-1 jrfl nef gene with the above mentioned modifications is disclosed herein as SEQ ID NO:13, as follows:

30

GATCTGCCAC CATGGCCGGC AAGTGGTCCA AGAGGTCCGT GCCCGGCTGG TCCACCGTGA
GGGAGAGGAT GAGGAGGGCC GAGCCCGCCG CCGACAGGGT GAGGAGGACC GAGCCCGCCG
CCGTGGGCGT GGGCGCCGTG TCCAGGGACC TGGAGAAGCA CGGCGCCATC ACCTCCTCCA
ACACCGCCGC CACCAACGCC GACTGCGCCT GGCTGGAGGC CCAGGAGGAC GAGGAGGTGG
GCTTCCCCGT GAGGCCCCAG GTGCCCCTGA GGCCCATGAC CTACAAGGGC GCCGTGGACC
TGTCCCACTT CCTGAAGGAG AAGGGCGGCC TGGAGGGCCT GATCCACTCC CAGAAGAGGC
AGGACATCCT GGACCTGTGG GTGTACCACA CCCAGGGCTA CTTCCCCGAC TGGCAGAACT
ACACCCCCGG CCCCGGCATC AGGTTCCCCC TGACCTTCGG CTGGTGCTTC AAGCTGGTGC
CCGTGGAGCC CGAGAAGGTG GAGGAGGCCA ACGAGGGCGA GAACAACTGC GCCGCCCACC
CCATGTCCCA GCACGGCATC GAGGACCCCG AGAAGGAGGT GCTGGAGTGG AGGTTCGACT
CCAAGCTGGC CTTCCACCAC GTGGCCAGGG AGCTGCACCC CGAGTACTAC AAGGACTGCT
AAAGCCCGGG C (SEQ ID NO:13).

The open reading frame of SEQ ID NO:13 encodes Nef (G2A,LLAA), disclosed herein as SEQ ID NO:14, as follows:

10

30

35

Met Ala Gly Lys Trp Ser Lys Arg Ser Val Pro Gly Trp Ser Thr Val 15 Arg Glu Arg Met Arg Arg Ala Glu Pro Ala Ala Asp Arg Val Arg Arg Thr Glu Pro Ala Ala Val Gly Val Gly Ala Val Ser Arg Asp Leu Glu Lys His Gly Ala Ile Thr Ser Ser Asn Thr Ala Ala Thr Asn Ala Asp Cys Ala Trp Leu Glu Ala Gln Glu Asp Glu Glu Val Gly Phe Pro Val 20 Arg Pro Gln Val Pro Leu Arg Pro Met Thr Tyr Lys Gly Ala Val Asp Leu Ser His Phe Leu Lys Glu Lys Gly Gly Leu Glu Gly Leu Ile His Ser Gln Lys Arg Gln Asp Ile Leu Asp Leu Trp Val Tyr His Thr Gln Gly Tyr Phe Pro Asp Trp Gln Asn Tyr Thr Pro Gly Pro Gly Ile Arg Phe Pro Leu Thr Phe Gly Trp Cys Phe Lys Leu Val Pro Val Glu Pro Glu Lys Val Glu Glu Ala Asn Glu Gly Glu Asn Asn Cys Ala Ala His 25 Pro Met Ser Gln His Gly Ile Glu Asp Pro Glu Lys Glu Val Leu Glu Trp Arg Phe Asp Ser Lys Leu Ala Phe His His Val Ala Arg Glu Leu His Pro Glu Tyr Tyr Lys Asp Cys Ser (SEQ ID NO:14).

An additional embodiment of the present invention relates to another DNA molecule encoding optimized HIV-1 Nef wherein the amino terminal myristylation site and dileucine motif have been deleted, as well as comprising a tPA leader peptide. This DNA molecule, opt tpanef (LLAA) comprises an open reading frame which encodes a Nef protein containing a tPA leader sequence fused to amino acid residue 6-216 of HIV-1 Nef (jfrl), wherein Leu-174 and Leu-175 are substituted with Ala-174 and Ala-175 (Ala-195 and Ala-196 in this tPA-based fusion protein). The nucleotide

sequence comprising the open reading frame of opt tpanef (LLAA) is disclosed herein as SEQ ID NO:15, as shown below:

CATGGATGCA ATGAAGAGAG GGCTCTGCTG TGTGCTGCTG CTGTGTGGAG CAGTCTTCGT TTCGCCCAGC GAGATCTCCT CCAAGAGGTC CGTGCCCGGC TGGTCCACCG TGAGGGAGAG GATGAGGAGG GCCGAGCCCG CCGCCGACAG GGTGAGGAGG ACCGAGCCCG CCGCCGTGGG CGTGGGCGCC GTGTCCAGGG ACCTGGAGAA GCACGGCGCC ATCACCTCCT CCAACACCGC CGCCACCAAC GCCGACTGCG CCTGGCTGGA GGCCCAGGAG GACGAGGAGG TGGGCTTCCC CGTGAGGCCC CAGGTGCCCC TGAGGCCCAT GACCTACAAG GGCGCCGTGG ACCTGTCCCA CTTCCTGAAG GAGAAGGGCG GCCTGGAGGG CCTGATCCAC TCCCAGAAGA GGCAGGACAT CCTGGACCTG TGGGTGTACC ACACCCAGGG CTACTTCCCC GACTGGCAGA ACTACACCCC CGGCCCCGGC ATCAGGTTCC CCCTGACCTT CGGCTGGTGC TTCAAGCTGG TGCCCGTGGA GCCCGAGAAG GTGGAGGAGG CCAACGAGGG CGAGAACAAC TGCGCCGCCC ACCCCATGTC CCAGCACGC ATCGAGGACC CCGAGAAGGA GGTGCTGGAG TGGAGGTTCG ACTCCAAGCT GGCCTTCCAC CACGTGGCCA GGGAGCTGCA CCCCGAGTAC TACAAGGACT GCTAAAGCCC (SEQ ID NO:15).

10

15

35

The open reading frame of SEQ ID NO:7 encoding tPA-Nef (LLAA), disclosed herein as SEQ ID NO:16, is as follows:

Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Cys Gly Ala Val Phe Val Ser Pro Ser Glu Ile Ser Ser Lys Arg Ser Val Pro 20 Gly Trp Ser Thr Val Arg Glu Arg Met Arg Arg Ala Glu Pro Ala Ala Asp Arg Val Arg Arg Thr Glu Pro Ala Ala Val Gly Val Gly Ala Val Ser Arg Asp Leu Glu Lys His Gly Ala Ile Thr Ser Ser Asn Thr Ala Ala Thr Asn Ala Asp Cys Ala Trp Leu Glu Ala Gln Glu Asp Glu Glu Val Gly Phe Pro Val Arg Pro Gln Val Pro Leu Arg Pro Met Thr Tyr Lys Gly Ala Val Asp Leu Ser His Phe Leu Lys Glu Lys Gly Gly Leu 25 Glu Gly Leu Ile His Ser Gln Lys Arg Gln Asp Ile Leu Asp Leu Trp Val Tyr His Thr Gln Gly Tyr Phe Pro Asp Trp Gln Asn Tyr Thr Pro Gly Pro Gly Ile Arg Phe Pro Leu Thr Phe Gly Trp Cys Phe Lys Leu Val Pro Val Glu Pro Glu Lys Val Glu Glu Ala Asn Glu Gly Glu Asn 30 Asn Cys Ala Ala His Pro Met Ser Gln His Gly Ile Glu Asp Pro Glu Lys Glu Val Leu Glu Trp Arg Phe Asp Ser Lys Leu Ala Phe His His Val Ala Arg Glu Leu His Pro Glu Tyr Tyr Lys Asp Cys (SEQ ID NO:16). An adenoviral vector of the present invention may comprise a DNA sequence, regardless of codon usage, which expresses a wild type or modified Nef protein as described herein, including but not limited to modified Nef proteins which comprise a deletion or substitution of Gly 2, a deletion of substitution of Leu 174 and Leu 175

and/or inclusion of a leader sequence. Therefore, partial or fully codon optimized DNA vaccine expression vector constructs are preferred since such constructs should result in increased host expression. However, it is within the scope of the present invention to utilize "non-codon optimized" versions of the constructs disclosed herein, especially modified versions of HIV Nef which are shown to promote a substantial cellular immune response subsequent to host administration.

Figure 20A-C show nucleotide sequences at junctions between nef coding sequence and plasmid backbone of nef expression vectors V1Jns/nef (Figure 20A), V1Jns/nef(G2A,LLAA) (Figure 20B), V1Jns/tpanef (Figure 20C) and V1Jns/tpanef(LLAA) (Figure 20C, also). 5' and 3' flanking sequences of codon optimized nef or codon optimized nef mutant genes are indicated by bold/italic letters; nef and nef mutant coding sequences are indicated by plain letters. Also indicated (as underlined) are the restriction endonuclease sites involved in construction of respective nef expression vectors. V1Jns/tpanef and V1Jns/tpanef(LLAA) have identical sequences at the junctions.

Figure 21 shows a schematic presentation of nef and nef derivatives. Amino acid residues involved in Nef derivatives are presented. Glycine 2 and Leucine 174 and 175 are the sites involved in myristylation and dileucine motif, respectively.

20 EXAMPLE 19

5

10

15

25

30

35

MRKAd5Pol Construction and Virus Rescue

Construction of vector: shuttle plasmid and pre-adenovirus plasmid - Key steps performed in the construction of the vectors, including the pre-adenovirus plasmid denoted MRKAd5pol, is depicted in Figure 22. Briefly, the adenoviral shuttle vector for the full-length inactivated HIV-1 pol gene is as follows. The vector MRKpdelE1(Pac/pIX/pack450)+CMVmin+BGHpA(str.) is a derivative of the shuttle vector used in the construction of the MRKAd5gag adenoviral pre-plasmid. The vector contains an expression cassette with the hCMV promoter (no intronA) and the bovine growth hormone polyadenylation signal. The expression unit has been inserted into the shuttle vector such that insertion of the gene of choice at a unique BgIII site will ensure the direction of transcription of the transgene will be Ad5 E1 parallel when inserted into the MRKpAd5(E1-/E3+)Cla1 (or MRKpAdHVE3) preplasmid. The vector, similar to the original shuttle vector contains the Pac1 site, extension to the packaging signal region, and extension to the pIX gene. The synthetic full-length codon-optimized HIV-1 pol gene was isolated directly from the plasmid pV1Jns-HIV-pol-inact(opt). Digestion of this plasmid with BgI II releases the pol

gene intact (comprising a codon optimized IA pol sequence as disclosed in SEQ ID NO:3). The pol fragment was gel purified and ligated into the MRKpdelE1(Pac/pIX/pack450)+CMVmin+BGHpA(str.) shuttle vector at the BgIII site. The clones were checked for the correct orientation of the gene by using restriction enzymes DraIII/Not1. A positive clone was isolated and named MRKpdel+hCMVmin+FL-pol+bGHpA(s). The genetic structure of this plasmid was verified by PCR, restriction enzyme and DNA sequencing. The pre-adenovirus plasmid was constructed as follows. Shuttle plasmid MRKpdel+hCMVmin+FLpol+bGHpA(S) was digested with restriction enzymes Pac1 and Bst1107 I (or its isoschizomer, BstZ107 I) and then co-transformed into E. coli strain BJ5183 with linearized (Cla1 digested) adenoviral backbone plasmid, MRKpAd(E1-/E3+)Cla1. The resulting pre-plasmid originally named MRKpAd+hCMVmin+FLpol+bGHpA(S)E3+ is now referred to as "pMRKAd5pol". The genetic structure of the resulting pMRKAd5pol was verified by PCR, restriction enzyme and DNA sequence analysis. The vectors were transformed into competent E. coli XL-1 Blue for preparative production. The recovered plasmid was verified by restriction enzyme digestion and DNA sequence analysis, and by expression of the pol transgene in transient transfection cell culture. The complete nucleotide sequence of this pMRKAd5HIV-1pol adenoviral vector is shown in Figure 26 A-AO.

10

15

20

25

30

35

Generation of research-grade recombinant adenovirus - The pre-adenovirus plasmid, pMRKAd5pol, was rescued as infectious virions in PER.C6® adherent monolayer cell culture. To rescue infectious virus, 12 μ g of pMRKAd5pol was digested with restriction enzyme PacI (New England Biolabs) and 3.3 μ g was transfected per 6 cm dish of PER.C6® cells using the calcium phosphate coprecipitation technique (Cell Phect Transfection Kit, Amersham Pharmacia Biotech Inc.). PacI digestion releases the viral genome from plasmid sequences allowing viral replication to occur after entry into PER.C6® cells. Infected cells and media were harvested 6 -10 days post-transfection, after complete viral cytopathic effect (CPE) was observed. Infected cells and media were stored at \leq -60°C. This pol containing recombinant adenovirus is referred to herein as "MRKAd5pol". This recombinant adenovirus expresses an inactivated HIV-1 Pol protein as shown in SEQ ID NO:6.

EXAMPLE 20

MRKAd5Nef Construction and Virus Rescue

Construction of vector: shuttle plasmid and pre-adenovirus plasmid - Key steps performed in the construction of the vectors, including the pre-adenovirus

plasmid denoted MRKAd5nef, is depicted in Figure 23. Briefly, as shown in Example 19 above, the vector

MRKpdelE1(Pac/pIX/pack450)+CMVmin+BGHpA(str.) is the shuttle vector used in the construction of the MRKAd5gag adenoviral pre-plasmid. It has been modified to contain the *Pac*1 site, extension to the packaging signal region, and extension to the pIX gene. It contains an expression cassette with the hCMV promoter (no intronA) and the bovine growth hormone polyadenylation signal. The expression unit has been inserted into the shuttle vector such that insertion of the gene of choice at a unique *Bgl*11 site will ensure the direction of transcription of the transgene will be Ad5 E1 parallel when inserted into the MRKpAd5(E1-/E3+)Cla1 pre-plasmid. The synthetic full-length codon-optimized HIV-1 nef gene was isolated directly from the plasmid pV1Jns/nef (G2A,LLAA). Digestion of this plasmid with *Bgl*11 releases the pol gene intact, which comprises the nucleotide sequence as disclosed in SEQ ID NO:13. The nef fragment was gel purified and ligated into the

10

30

35

15 MRKpdelE1+CMVmin+BGHpA(str.) shuttle vector at the Bgl11 site. The clones were checked for correction orientation of the gene by using restriction enzyme Scal. A positive clone was isolated and named MRKpdelE1hCMVminFL-nefBGHpA(s). The genetic structure of this plasmid was verified by PCR, restriction enzyme and DNA sequencing. The pre-adenovirus plasmid was constructed as follows. Shuttle plasmid MRKpdelE1hCMVminFL-nefBGHpA(s) was digested with restriction 20 enzymes Pac1 and Bst1107 I (or its isoschizomer, BstZ107 I) and then co-transformed into E. coli strain BJ5183 with linearized (Cla1 digested) adenoviral backbone plasmid, MRKpAd(E1/E3+)Cla1. The resulting pre-plasmid originally named MRKpdelE1hCMVminFL-nefBGHpA(s) is now referred to as "pMRKAd5nef". The 25 genetic structure of the resulting pMRKAd5nef was verified by PCR, restriction enzyme and DNA sequence analysis. The vectors were transformed into competent E. coli XL-1 Blue for preparative production. The recovered plasmid was verified by restriction enzyme digestion and DNA sequence analysis, and by expression of the nef transgene in transient transfection cell culture. The complete nucleotide sequence

Generation of research-grade recombinant adenovirus - The pre-adenovirus plasmid, pMRKAd5nef, was rescued as infectious virions in PER.C6[®] adherent monolayer cell culture. To rescue infectious virus, 12 μg of pMRKAdnef was digested with restriction enzyme Pac1 (New England Biolabs) and 3.3 μg was transfected per 6 cm dish of PER.C6[®] cells using the calcium phosphate coprecipitation technique (Cell Phect Transfection Kit, Amersham Pharmacia Biotech

of this pMRKAd5HIV-1nef adenoviral vector is shown in Figure 27A-AM.

Inc.). Pac1 digestion releases the viral genome from plasmid sequences allowing viral replication to occur after entry into PER.C6®cells. Infected cells and media were harvested 6-10 days post-transfection, after complete viral cytopathic effect (CPE) was observed. Infected cells and media were stored at \leq -60°C. This nef containing recombinant adenovirus is now referred to as "MRKAd5nef".

EXAMPLE 21

5

10

15

20

25

30

35

Construction of Murine CMV Promoter Containing Shuttle Vectors for Inactivated Pol and Nef/G2A,LLAA

The murine CMV (mCMV) was amplified from the plasmid pMH4 (supplied by Frank Graham, McMaster University) using the primer set: mCMV (Not I) Forward: 5'-ATA AGA ATG CGG CCG CCA TAT ACT GAG TCA TTA GG-3' (SEQ ID NO: 20); mCMV (Bgl II)Reverse: 5'-AAG GAA GAT CTA CCG ACG CTG GTC GCG CCT C-3' (SEQ ID NO:21). The underlined nucleotides represent the Not I and the $Bgl \Pi$ sites respectively for each primer. This PCR amplicon was used for the construction of the mCMV shuttle vector containing the transgene in the E1 parallel orientation. The hCMV promoter was removed from the original shuttle vector (containing the hCMV-gag-bGHpA transgene in the E1 parallel orientation) by digestion with Not I and Bgl II. The mCMV promoter (Not I/Bgl II digested PCR product) was inserted into the shuttle vector in a directional manner. The shuttle vector was then digested with $Bgl ext{ II}$ and the gag reporter gene ($Bgl ext{ II}$ fragment) was re-inserted back into the shuttle vector. Several clones were screened for correct orientation of the reporter gene. For the construction of the mCMV-gag in the E1 antiparallel orientation, the mCMV promoter was amplified from the plasmid pMH4 using the following primer set: mCMV (Asc I) Forward: 5'- ATA AGA ATG GCG CGC CAT ATA CTG AGT CAT TAG G (SEQ ID NO:22); mCMV (Bgl II) Reverse: 5' AAG GAA GAT CTA CCG ACG CTG GTC GCG CCT C (SEQ ID NO:23). The underlined nucleotides represent the Asc I and Bgl II sites, respectively for each primer. The shuttle vector containing the hCMV-gag transgene in the E1 antiparallel orientation was digested with Asc1 and Bgl11 to remove the hCMV-gag portion of the transgene. The mCMV promoter (Asc1/Bgl11 digested PCR product) was inserted into the shuttle vector in a directional manner. The vector was then digested with Bgl11 and the gag reporter gene (Bgl11 fragment) was re-inserted. Several clones were screened for correct orientation of the reporter gene. For each of the full length IA pol and full length nef/G2A,LLAA genes, cloning was performed using the unique

Bgl II site within the mCMV-bGHpA shuttle vector. The pol and nef genes were excised from their respective pV1Jns plasmids by Bgl II digestion.

5

10

15

35

EXAMPLE 22

Construction of mCMV Full Length Inactivated Pol and Full Length nef/G2A.LLAA Adenovectors

Each of these transgenes of Example 21 were inserted into the modified shuttle vector in both the E1 parallel and E1 anti-parallel orientations. Pac1 and BstZ110I digestion of each shuttle vector was performed and each specific transgene fragment containing the flanking Ad5 sequences was isolated and co-transformed with Cla I digested MRKpAd5(E3+) or MRKpAd5(E3-) adenovector plasmids via bacterial homologous recombination in BJ5183 E. coli cells. Recombinant preplasmid adenovectors containing the various transgenes in both the E3- and E3+ versions (and in the E1 parallel and E1 antiparallel orientations) were subsequently prepared in large scale following transformation into XL-1 Blue E. coli cells and analyzed by restriction analysis and sequencing.

EXAMPLE 23

Construction of hCMV-tpa-nef (LLAA) Adenovector

The tpa-nef gene was amplified out from GMP grade pV1Jns-tpanef (LLAA) vector using the primer sets: Tpanef (BamHI) F 5'-ATT GGA TCC ATG GAT GCA ATG AAG AGA GGG (SEQ ID 24); Tpanef (BamHI) R 5'-ATA GGA TCC TTA GCA GTC CTT GTA GTA CTC G (SEQ ID NO:25). The resulting PCR product was digested with BamHI, gel purified and cloned into the Bgl II site of MRKAd5CMV-bGHpA shuttle vector (Bgl II digested and calf intestinal phosphatase treated). Clones containing the tpanef (LLAA) gene (see SEQ ID NO:15 for complet coding region) in the correct orientation with respect to the hCMV promoter were selected following Sca I digestion. The resulting MRKAd5tpanef shuttle vector was digested with Pac I and Bst Z1101 and cloned into the E3+ MRKAd5 adenovector via bacterial homologous recombination techniques.

EXAMPLE 24

Immunogenicity of MRKAd5pol and MRKAd5nef Vaccine

Materials and Methods - Rodent Immunization - Groups of N=10 BALB/c

mice were immunized i.m. with the following vectors: (1) MRKAd5hCMV-IApol

(E3+) at either 10^7 vp and 10^9 vp; and (2) MRKAd5hCMV-IApol (E3-) at either

10^7 vp and 10^9 vp. At 7 weeks post dose, 5 of the 10 mice per cohort were boosted with the same vector and dose they initially received. At 3 weeks post the second does, sera and spleens were collected from all the animals for RT ELISA and IFNg ELIspot analyses, respectively. For all rodent immunizations, the Ad5 vectors were diluted in 5 mM Tris, 5% sucrose, 75 mM NaCl, 1 mM MgCl2, 0.005% polysorbate 80, pH 8.0. The total dose was injected to both quadricep muscles in 50 µL aliquots using a 0.3-mL insulin syringe with 28-1/2G needles (Becton-Dickinson, Franklin Lakes, NJ).

Groups of N=10 C57/BL6 mice were immunized i.m. with the following vectors: (1) MRKAd5hCMV-nef(G2A,LLAA) (E3+) at either 10^7 vp and 10^9 vp; (2) MRKAd5mCMV-nef(G2A,LLAA) (E3+) at either 10^7 vp and 10^9 vp; and (3) MRKAd5mCMV-tpanef(LLAA) (E3+) at either 10^7 vp and 10^9 vp. At 7 weeks post dose, 5 of the 10 mice per cohort were boosted with the same vector and dose they initially received. At 3 weeks post the second does, sera and spleens were collected from all the animals for RT ELISA and IFNg ELIspot analyses, respectively.

Non-human Primate immunization - Cohorts of 3 rhesus macaques (2-3 kg) were vaccinated with the following Ad vectors: (1) MRKAd5hCMV-IApol (E3+) at either 10^9 vp and 10^11 vp dose; and (2) MRKAd5hCMV-IApol (E3-) at either 10^9 vp and 10^11 vp; (3) MRKAd5hCMV-nef(G2A,LLAA) (E3+) at either 10^9 vp and 10^11 vp; and (4) MRKAd5mCMV-nef(G2A,LLAA) (E3+) at either 10^9 vp and 10^11 vp. The vaccine was administered to chemically restrained monkeys (10 mg/kg ketamine) by needle injection of two 0.5 mL aliquots of the Ad vectors (in 5 mM Tris, 5% sucrose, 75 mM NaCl, 1 mM MgCl₂, 0.005% polysorbate 80, pH 8.0) into both deltoid muscles. The animals were immunized twice at a 4 week interval (T=0, 4 weeks).

Murine anti-RT and anti-nef ELISA - Anti-RT titers were obtained following standard secondary antibody-based ELISA. Maxisorp plates (NUNC, Rochester; NY) were coated by overnight incubation with 100 μL of 1 μg/mL HIV-1 RT protein (Advanced Biotechnologies, Columbia, MD) in PBS. For anti-nef ELISA, 100 uL of 1 ug/mL HIV-1 nef (Advanced Biotechnologies, Columbia, MD) was used to coat the plates. The plates were washed with PBS/0.05% Tween 20 using Titertek MAP instrument (Hunstville, AL) and incubated for 2 h with 200 μL/well of blocking solution (PBS/0.05% tween/1% BSA). An initial serum dilution of 100-fold was performed followed by 4-fold serial dilution. 100-μL aliquots of serially diluted samples were added per well and incubated for 2 h at room temperature. The plates

were washed and 100 μ L of 1/1000-diluted HRP-rabbit anti-mouse IgG (ZYMED, San Francisco, CA) were added with 1 h incubation. The plates were washed thoroughly and soaked with 100 μ L 1,2-phenylenediamine dihydrochloride/hydrogen peroxide (DAKO, Norway) solution for 15 min. The reaction was quenched by adding 100 μ L of 0.5M H₂SO4 per well. OD₄₉₂ readings were recorded using Titertek Multiskan MCC/340 with S20 stacker. Endpoint titers were defined as the highest serum dilution that resulted in an absorbance value of greater than or equal to 0.1 OD₄₉₂ (2.5 times the background value).

Non-human primate and murine ELIspot assays - The enzyme-linked immuno-spot (ELISpot) assay was utilized to enumerate antigen-specific INFγ-secreting cells from mouse spleens (Miyahira, et al.1995, J. Immunol. Methods 181:45-54) or macaque PBMCs. Mouse spleens were pooled from 5 mice/cohort and single cell suspensions were prepared at 5x10⁶/mL in complete RPMI media (RPMI1640, 10% FBS, 2mM L-glutamine, 100U/mL Penicillin, 100 u/mL streptomycin, 10 mM Hepes, 50 uM β-ME). Rhesus PBMCs were prepared from 8-15 mL of heparinized blood following standard Ficoll gradient separation (Coligan, et al, 1998, Current Protocols in Immunology. John Wiley & Sons, Inc.). Multiscreen opaque plates (Millipore, France) were coated with 100 μL/well of either 5 μg/mL purified rat anti-mouse IFN-γ IgG1, clone R4-6A2 (Pharmingen, San Diego, CA), or 15 ug/mL mouse anti-human IFN-γ IgG2a (Cat. No. 1598-00, R&D Systems, Minneapolis, MN) in PBS at 4°C overnight for murine or monkey assays, respectively. The plates were washed with PBS/penicillin/streptomycin and blocked with 200 μL/well of complete RPMI media for 37 °C for at least 2 h.

To each well, 50 μL of cell samples (4-5x10⁵ cells per well) and 50 μL of the antigen solution were added. To the control well, 50 μL of the media containing DMSO were added; for specific responses, either selected peptides or peptide pools (4 ug/mL per peptide final concentration) were added. For BALB/c mice immunized with the pol constructs, stimulation was conducted using a pool of CD4⁺-epitope containing 20-mer peptides (aa21-40, aa411-430, aa641-660, aa731-750, aa771-790) or a pool of CD8⁺-epitope containing peptides (aa201-220, aa311-330, aa781-800). For C57/BL6 mice immunized with the nef construct, either aa51-70 (CD8⁺ T cell epitope) or aa81-100 (CD4⁺) peptide derived from the nef sequence was added for specific stimulation. In monkeys, the responses against pol were evaluated using two pools (L and R) of 20-aa peptides that encompass the entire pol sequence and overlap by 10 amino acids. In monkeys vaccinated with the nef constructs, a single pool containing 20-mer peptides covering the entire HIV-1 nef sequence and overlapping

by 10 aa was used. Each sample/antigen mixture was performed in triplicate wells for murine samples or in duplicate wells for rhesus PBMCs. Plates were incubated at 37°C, 5% CO₂, 90% humidity for 20-24 h. The plates were washed with PBS/0.05% Tween 20 and incubated with 100 μL/well of either 1.25 μg/mL biotin-conjugated rat anti-mouse IFN-γ mAb, clone XMG1.2 (Pharmingen) or of 0.1 ug/mL biotinylated anti-human IFN-gamma goat polyclonal antibody (R&D Systems) at 4°C overnight. The plates were washed and incubated with 100 μL/well 1/2500 dilution of strepavidin-alkaline phosphatase conjugate (Pharmingen) in PBS/0.005% Tween/5% FBS for 30 min at 37 °C. Spots were developed by incubating with 100 μL/well 1-step NBT/BCIP (Pierce Chemicals) for 6-10 min. The plates were washed with water and allowed to air dry. The number of spots in each well was determined using a dissecting microscope and the data normalized to 10⁶ cell input.

Non-human Primate anti-RT ELISA - The pol-specific antibodies in the monkeys were measured in a competitive RT EIA assay, wherein sample activity is determined by the ability to block RT antigen from binding to coating antibody on the plate well. Briefly, Maxisorp plates were coated with saturating amounts of pol positive human serum (#97111234). 250 uL of each sample is incubated with 15 uL of 266 ng/mL RT recombinant protein (in RCM 563, 1% BSA, 0.1% tween, 0.1% NaN₃) and 20 uL of lysis buffer (Coulter p24 antigen assay kit) for 15 min at room temperature. Similar mixtures are prepared using serially diluted samples of a standard and a negative control which defines maximum RT binding. 200 uL/well of each sample and standard were added to the washed plate and the plate incubated 16-24 h at room temperature. Bound RT is quantified following the procedures described in Coulter p24 assay kit and reported in milliMerck units per mL arbitrarily defined by the chosen standard.

Results - Rodent Studies - BALB/c mice (n=5 mice/cohort) were immunized once or twice with varying doses of MRKAd5hCMV-IApol(E3+) and MRKAd5hCMV-IApol(E3-). At 3 weeks after the second dose, Anti-pol IgG levels were determined by an ELISA assay using RT as a surrogate antigen. Cellular response were quantified via IFNγ ELISpot assay against pools of pol-epitope containing peptides. The results of these assays are summarized in Table 10. The results indicate that the mouse vaccinees exhibited detectable anti-RT IgGs with an adenovector dose as low as 10⁴7 vp. The humoral responses are highly dosedependent and are boostable with a second immunization. One or two doses of either pol vectors elicit high frequencies of antigen-specific CD4⁺ and CD8⁺ T cells; the responses are weakly dose-dependent but are boostable with a second immunization.

Table 10. Immunogenicity of MRKAd5pol Vectors in BALB/c mice.

				Ar	ti-RT IgG Tite	ers"	8	FC/10^6 cell	ls°
Group	Vaccine	Dose	No. of Doses	GMT	+SE	-SE	Medium	CD4+ peptide pool	CD8+ peptide pool
1	MRKAd5hCMVFLpol (E3+)	10^7 vp	2 1	310419 919	301785 372	153020 265	1(1) 1(1)	75(4) 72(9)	2313(67) 533(41)
2	MRKAd5hCMVFLpol (E3+)	10^9 vp	2 1	1638400 ^b 713155	0 528520	0 303555	2(2) 1(1)	114(9) 48(7)	2063(182) 733(89)
3	MRKAd5hCMVFLpol (E3-)	10^7 vp	2	310419 6400	386218 14013	172097 4393	0(0) 10(8)	223(7) 141(21)	2607(27) 409(28)
4	MRKAd5hCMVFLpol (E3-)	10 ^9 vp	2	1638400 ^b 1241675 ^b	0 396725	0 300661	1(1) 0(0)	160(13) 39(13)	2385(11) 833(83)
5	Naïve	none	none	57	9	7	9(2)	11(4)	10(1)

^{*}GMT, geometric mean titer of the cohort of 5 mice; SE, standard error of the gemetric mean

5 C57/BL6 mice were immunized once or twice with varying doses of MRKAd5hCMV-nef(G2A,LLAA) (E3+), MRKAd5mCMV-nef(G2A,LLAA) (E3+) at either 10^7 vp and(3) MRKAd5mCMV-tpanef(LLAA) (E3+) at either 10^7 vp and 10^9 vp. The immune response were analyzed using similar protocols and the results are listed in Table 11. While anti-nef IgG responses could not be detected in this model system with any of the constructs, there are strong indications of a cellular immunity generated against nef using the ELIspot assay.

Table 11. Immunogenicity of MRKAd5nef Vectors in C57/BL6 mice.

				Ar	ti-nef lgG Tite	ers"	S	FC/10^6 cell	s ^b
Group	Vaccine	Dose	No. of Doses	GMT	+SE	-SE	Medlum	aa51-70 CD8+	aa81-100 CD4+
1	MRKAdShCMVFLnef (E3+)	10^7 vp	1	174 132	70 42	50 32	1(1) 0(0)	23(1) 0(0)	1(1) 0(0)
2	MRKAd5hCMVFLnef (E3+)	10^9 vp	2	174 132	70 42	50 32	0(0) 1(1)	61(7) 62(7)	4(2) 3(1)
3	MRKAdSmCMVFLnef (E3+)	10^7 vp	2	132 115	42 46	32 33	3(1) 3(2)	15(5) 3(2)	5(2) 4(2)
4	MRKAd5mCMVFLnef (E3+)	10^9 vp	2	132 132	42 42	32 32	4(2) 2(1)	83(13) 29(2)	5(1) 4(0)
5	MRKAd5mCMVtpanef(E3+)	10^7 vp	2 1	132 100	42 0	32 0	3(2) 3(1)	14(2) 13(4)	5(1) 10(3)
6	MRKAd5mCMVtpanef(E3+)	10^9 vp	2 1	230 115	170 46	98 33	3(2) 7(1)	145(29) 151(14)	4(0) 10(0)
7	Naïve	none	none	152	78	52 ·	21(2)	18(6)	26(3)

^{*}GMT, geometric mean titer of the cohort of 5 mice; SE, standard error of the gemetric mean

15

Monkey Studies - Cohorts of 3 rhesus macaques were immunized with 2 doses of MRKAd5hCMV-IApol(E3+) and MRKAd5hCMV-IApol(E3-). The number of antigen-specific T cells (per million PBMCs) were enumerated using one of two

Near or at the upper limit of the serial dilution; hence, could be greater than this value

[&]quot;No. of Spot-forming Cells per million splechoytes; mean values of triplicates are reported along with standard errors in parenthesis.

No. of spot-forming cells per million splecnoytes; mean values of triplicates are reported along with standard errors in parenthesis.

peptide pools (L and R) that cover the entire pol sequence; the results are listed in Table 12. Moderate-to-strong T cell responses were detected in the vaccinees using either constructs even at a low dose of 10^9 vp. Longitudinal analyses of the anti-RT antibody titers in the animals suggest that the pol transgene product is expressed efficiently to elicit a humoral response (Table 13). It would appear that generally higher immune responses were observed in animals that received the E3- construct compared to the E3+ virus.

Table 12. Pol-specific T Cell Responses in MRKAd5pol Immunized Rhesus

10 Macaques.

Vaccine (T=0,4 wks)	Monk #		Prebieed	1		T=4			T=7			T¤16	
		Mock	Pol L	Pol R	Mock	Pol L	Pol R	Mock	Pol L	Pol R	Mock	Pol L	Pol R
MRKAd5hCMV-lAppl(E3+)	99C100	1	0	0	1	38	31	0	52	146	0	49	715
10^11 vp	99C215	1 1	2	2	10	98	249	1 1	109	305	22	88	250
	99D201	5	5	4	6	149	95	0	40	35	0	35	18
MRKAc5hCMV-IApol(E3+)	99D212	0	2	0	4	331	114	0	58	14	0	6	6
10/9 vp	99D18D	0	4	2	0	19	192	4	36	156	5	38	106
	990201	8	5	21	6	62	82	0	18	32	ו	14	65
MRK Ad5hCMV+lApd(E3-)	99D239	5	2	2	20	82	172	1	68	114	9	21	40
10^11 vp	99C186	4	12	6	5	120	421	2	271	489	16	875	530
- · · · •	99C084	1	8	8	8	84	484	0	14	236	1	24	264
MRK Ad5hCMV-I Apd (E3-)	CC7C	10	10	8	12	724	745	4	322	376	4	188	176
10/9 vo	CDIG	2	0	1	5	474	468	0	232	212	0	101	121
	CD11	6	6	12	10	98	110	5	60	80	8	25	34
Nove	083Q	nd	nd	nd	nd	nd	nd	4	2	2	2		2

nd, not determined Reported are SFC per million PBMCs; mean of duplicate wells.

Table 13. Anti-RT Ig Levels in MRKAd5pol Immunized macaques.

/mL			
T =4	T =7	T=12	T=16
61	1999	5928	4768
81	1541	2356	2767
53	336	539	387
10	40	49	68
<10	36	_79	93
<10	37	71	76
44	460	1234	1015
21	233	480	345
235	2637	2858	1626
32	175	306	235
20	140	273	419
15	112	149	237
	61 81 53 10 <10 <10 21 235	1 1 2 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3	T=4 T=7 T=12 61 1999 5928 81 1541 2356 53 336 539 10 40 49 <10

When rhesus macaques were immunized i.m. with two doses of MRKAd5nef constructs, vigorous T cell responses ranging from 100 to as high as 1100 per million were observed in 8 of 12 vaccinees (Table 14). The efficacies of the mCMV- and hCMV- driven nef constructs are comparable on the basis of the data generated thus far.

5

15

20

25

10 Table 14. Nef-specific T cell Responses in MRKAd5nef Immunized Rhesus Macaques.

Vaccine (T=0,4 wks)	Monk #	P	re	T:	=4	T:	=7	T=	:16
		Mock	Nef	Mock	Nef	Mock	Nef	Mock	Nef
MRKAd5hCMV-nef(G2A,LLAA) (E3+)	CD2D		4	31	440	4	368	1	251
10^11 vp	CC7B	0	0	2	521	٥	178	1	1522
·	CC61	2	9	31	112	0	108	11	100
MRKAd5hCMV-nef(G2A,LLAA) (E3+)	CC2K	9	9	6	52	0	35	0	15
10^9 vp	CD15	5	4	30	998	2	586	0	434
	CD16	6	1	6	1146	0	369	1	212
MRKAd5mCMV-nef(G2A,LLAA) (E3+)	99D191	1	5	4	614	0	298	2	419
10^11 vp	99D144	4	6	5	434	0	1100	2	932
	99C193	1 1	2	1	58	1	22	0	64
MRKAd5mCMV-nef(G2A,LLAA) (E3+)	99D224	1	11	14	231	1	125	0	70
10 ^9 vp	99D250	8	9	4	108	0	54	0	5
· ·	99C120	1	6	20	299	0	92	0	79
Naīve	083Q	nd	nd	18	22	4	5	2	-

EXAMPLE 25

Comparison of Clade B vs. Clade C T Cell Responses in HIV-Infected Subjects PBMC samples collected from two dozens of patients infected with HIV-1 in US were tested in ELISPOT assays with peptide pools of 20-mer peptides overlapping by 10 amino acids. Four different peptide pools were tested for cross-clade recognition, and they were either derived from a clade B-based isolate (gag H-b; nef-b) or a clade C-based isolate (gag H-c, nef-c). Data in Table 15 shows that T cells from these patients presumably infected with clade B HIV-1 could recognize clade C gag and nef antigens in ELISPOT assay. Correlation analysis further demonstrated that these T cell responses against clade C gag peptide pool were about 60% of the clade B counterpart (Figure 24), while the T cell responses against clade C nef were about 85% of the clade B counterpart (Figure 25). These results suggest that cellular immune responses generated in patients infected with clade B HIV-1 can recognize gag and nef antigens derived from clade C HIV-1. These data show that a HIV vaccine, such as a DNA or MRKAd5-based adenoviral vaccine expressing a clade B

gag and/or nef antigen will potentially have the ability to provide a prophylactic and/or therapetic advantage on a global scale.

5

Table 15
Responses Shown as the Number of gIFN-Secreting T Cells per Million PBMCs

subject	bleed date	gag epitope #	mock	gag H-b	gagH-c	nef-b	nef-c
*		from mapping)					
#100	19-Jul-99	12	10	3950	1385	1295	1300
#101	25-Jul-99	3	15	3885	1280	na	1020
#102	25-Jul-99	4	15	1740	850	1255	1785
#104	7-Jun-99	2	5	1355	1185	na	1060
#107	11-Oct-99	2	25	3305	2795	670	870
#405	11-Jul-99	2	15	4575	3180	1700	1500
#501	19-Jul-99	2	15	1100	570	3365	3460
#505	18-Jul-99	5	10	2145	1725	1235	na
#506	28-Feb-99	2	25	150	45	400	610
#701	28-Mar-99	5	30	7620	4775	3320	2780
#709	17-May-99	3	15	2785	1945	1090	1630
#710	24-May-99		5	1055	1080	2210	2140
					,		

10

EXAMPLE 26

Characterization and Production of MRKAd5pol and MRKAd5nef Vectors in Roller Bottles

Expansion of nef and pol Adenovectors - Nef and pol CsCl purified MRKAd5 seeds were used to infect roller bottles to produce P4 virus to be used as a seed for further experiments. P4 MRKAd5 pol and nef vectors were used to infect roller bottles at an MOI 280 vp/cell, except for hCMV-tpa-nef [E3+] which was infected at an MOI of 125 due to low titers of seed obtained at P4.

20

Table 16 Viral particle concentrations for P5 nef and pol adenovectors

Adenovector	AEX Titer (10 ¹⁰ vp/ml culture)	AEX Titer (10 ⁴ vp/cell)	Amplification Ratio
hCMV-FL-nef [E3+]	1.1	0.9	30
mCMV-FL-nef [E3+]	2.2	2.1	75
hCMV-tpa-nef [E3+]	0.07	0.1	5
mCMV-tpa-nef [E3+]	1.3	0.9	35
hCMV-FL-pol [E3+]	2.7	2.1	75
hCMV-FL-pol [E3-]	1.9	1.3	45

Roller Bottle Passaging - Passaging of the pol and nef constructs continued through passage seven. Cell-associated (freeze/thaw lysis) and whole broth (tritonlysis) titers obtained in all passages were very consistent. In general, MRKAd5pol is ca. 70% as productive as MRKAd5gag while MRKAd5nef is ca. 25% as productive as MRKAd5gag. Samples of P7 virus for both constructs were analyzed by V&CB by restriction digest analysis and did not show any rearrangements.

Table 17. Passage Six Viral Productivity for MRKAd5pol and MRKAd5nef

			0° cells/ml), ity (%) Harvest	Cell Passage Number	AEX Titer (Cell Associated) 1010 vp/ml culture	Titer 10' vp/cell	Amplification Ratio	Triton Lysis Titer 10 ¹⁰ vp/ml culture
hCMV-FL-nef [B3+]	pool	1.22, 85%		62	0.8	0.7	25	1.6
	1		0.99, 62%					
	2		1.10, 72%	}	}			
hCMV-FL-pol (E3+)	pool	1.42, 89%		62	4.5	3.2	115	7.0
	1		1.22, 70%					
	2		1.42, 74%					

15 Table 18. Passage Seven Viral Productivity for MRKAd5pol and MRKAd5nef

	ĺ		0° cells/ml), ity (%)	Cell Passage	AEX Titer (Cell Associated)	Titer	Amplification	Triton Lysis Titer
		Infection	Harvest	Number	1010 vp/ml culture	10 ⁴ vp/cell	Ratio	10 ¹⁰ vp/ml culture
hCMV-FL-nof [E3+]	Pool	1.33, 90%		66	1.0	0.8	29	2.1
	1		0.96, 70%	-				
	2		1.18,73%	ì	\		i	
hCMV-FL-pol [E3+]	Pool	0.90*, 90%		56	4.2	4.7	168	6.5
			1.18, 88%	•				
1	2		1.04, 80%					

MRKAd5nef and MRKAd5pol Viral Production Kinetics - A timecourse experiment was carried out in roller bottles to determine if the viral production kinetics of the MRKAd5pol and MRKAd5nef vectors were similar to those of

MRKAd5gag. PER.C6® cells in roller bottle cultures were infected at an MOI of 280 vp/cells with P5 MRKAd5pol, P5 MRKAd5nef and P7 MRKAd5gag; for each adenovector, two infected bottles were sampled at 24, 36, 48, and 60 hours post infection. In addition, two bottles were left unsampled until 48 hpi when they were harvested under the Phase I process conditions. The anion-exchange HPLC viral particle concentrations of the freeze-thaw recovered cell associated virus at the 24, 36,

48, and 60 hpi timepoints are shown in Figure 29A-B. The QPA titers show a similar trend (data not shown).

Comparison of hCMV- and mCMV-FL-nef - As the titers obtained with the MRKAd5nef construct (hCMV-FL-nef) were lower than those obtained with MRKAd5gag or MRKAd5pol, a viral productivity comparison experiment was performed with mCMV-FL-nef. For each of the two adenovectors (hCMV- and mCMV-FL-nef), two roller bottles were infected at an MOI of 280 vp/cell with passage five clarified lysate. The macroscopic and microscopic observations of the four roller bottles were identical at the time of harvest. Analysis of the clarified lysate produced indicated a higher viral particle concentration in the bottles infected with mCMV-FL-nef, as shown in Table 19. It is stipulated that the higher productivity with mCMV promoter driven nef vector is due to lower nef expression levels in PER.C6® cells- experiments are underway at V&CB to measure nef expression levels.

15

Table 19. Passage Six Viral Productivity Comparison of hCMV- and mCMV-FL-nef

	[Xv (10 ⁶ cells/ml), Viability (%)		Cell Passage	AEX Titer	Titer	Amplification	Triton Lysis Titer
		Infection	Harvest	Number	10 ¹⁰ vp/ml culture	104 vp/cell	Ratio	10 ¹⁰ vp/ml culture
hCMV-FL-nef	Pool	1.11, 91%		60	1.5	1.4	50	2.8
(MRKAd5nef)	1		1.23, 75%					
	2		1.34, 74%					
mCMV-FL-nef	Pool	1.11, 91%		60	2.3	2.1	75	4.6
	1		1.49, 84%					
	2		1.18, 77%	1				

20

25

EXAMPLE 27

Characterization and Large Scale Production of MRKAd5nef Virus in Bioreactors

Materials and Methods - The experiment of the present example was run twice under the following conditions: 36.5°C, DO 30%, pH 7.30, 150rpm agitation rate, no sparging, Life Technologies (Gibco, Invitrogen) 293 SFM II (with 6mM L-glutamine), 0.5M NaOH as base for pH control. During the first run (B20010115), two 10L stirred vessel bioreactors were inoculated with PER.C6® cells at a concentration of 0.2x106 cells/ml. Cells were grown until they reached a cell concentration of approximately 1x106 cells/ml. The cells were infected with uncloned MRKAd5nef (G2A,LLAA) at a MOI of 280 virus particles (vp)/cell. For the second batch (B20010202), the same procedure as the first run was used, except the cells

were infected with cloned MRAd5nef. During both runs, the bioreactors were harvested 48 hours post-infection. Samples were taken and virus concentrations were determined from whole broth (with triton lysis), supernatant, and cell pellets (3 X freeze/thaw) with the AEX and QPA assays. Metabolites were measured with BioProfile 250 throughout the process.

Table 20: Experimental Conditions

Temperature	36.5 ℃	
DO	30%	
PH	7.30	
Agitation	150 rpm	
Sparging	None	

Table 21: Virus source used for experiments.

10

15

5

Run	Batch ID	Cloned/Uncloned MRKAd5nef	MOI (vp/cells)
#1	B20010115-1	Uncloned	280
	B20010115-2	Uncloned	280
#2	B20010202-1	Cloned	280
	B20010202-2	Cloned	280

Results - Table 22 and 23 show an the ability to scale up production of MRKAd5nef by growth in a bioreactor.

Table 22: Virus Concentration as measured by the AEX assay

Run	Batch ID	Cloned/Uncloned	V	irus Concentration @	9 48hpi (1x1	10 ¹³ vp/L)			
L		MRKAd5nef	0.72 3.26 3.98 5.76 0.38 1.67 2.05 2.46						
#1	B20010115-1	Uncloned	0.72	3.26	3.98	5.76			
	B20010115-2	Uncloned	0.38	3.72 3.26 3.98 5.76 3.38 1.67 2.05 2.46					
#2	B20010202-1	Cloned	0.80	6.00	6.80	8.88			
	B20010202-2	Cloned	0.50	6.00	6.50	8.47			

Table 23: Virus Titers as measured by the QPA assay

Run	Batch ID	Cloned/Uncloned		Virus Concent	ration @ 48hpi	(1x10 ¹¹ IU/L)	
		MRKAd5nef	Whole	Supernatant	Clarified	Total	Triton
			Broth		Lysate		Lysate
#1	B20010115-1	Uncloned	0.13	1.12	1.76	2.88	11.28
	B20010115-2	Uncloned	0.14	0.73	1.54	2.27	5.86
#2	B20010202-1	Cloned	0.14	0.97	1.62	2.69	11.89
L	B20010202-2	Cloned	0.14	1.17	1.70	2.97	12.47

20

The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art

from the foregoing description. Such modifications are intended to fall within the scope of the appended claims.

EXAMPLE 28 MRKAd5HIV-1gag Boosting of DNA-Primed Animals

5

10

15

20

25

30

Groups of 3-5 rhesus macaques were immunized with (a) 5 mgs of V1Jns-Flgag (pVIJnsCMV(no intron)-FL-gag-bGHpA), (b) 5 mgs of V1Jns-Flgag formulated with 45 mgs of a non-ionic block copolymer CRL1005, or (c) 5 mgs of V1Jns-Flgag formulated with 7.5 mgs of CRL1005 and 0.6 mM benzalkonium chloride at weeks 0, 4, and 8. All animals received a single dose of 10e7 viral particles (vp) of the MRKAd5HIV-1gag at week 26. Note: 10e7 is too low to prime or boost effectively when used as a single modality (dose is selected to mimic preexposure to adenovirus); see Figure 32.

Blood samples were collected from all animals at several time points and peripheral blood mononuclear cells (PBMCs) were prepared using standard Ficoll method. The PBMCs were counted and analyzed for gamma-interferon secretion using the ELISpot assay (Table 24). For each monkey, the PBMCs were incubated overnight either in the absence (medium) or presence of a pool (called "gag H") of 50 20-aa long peptides that encompass the entire HIV-1 gag sequence.

The results indicate that MRKAd5HIV-1gag was very effective in boosting the T cell immune responses in these monkeys. At week 28 or 2 weeks after the viral boost, the number of gag-specific T cells per million PBMCs increased 2-48 fold compared to the levels observed at week 24 or 2 weeks prior to the boost.

The PBMCs were also analyzed by intracellular gamma-interferon staining prior to (at week 10) and after the MRKAd5gag boost (at week 30). The results for select animals are shown on Figure 31. The results indicate that (a) immunization with DNA/adjuvant formulation elicited T cell responses which can either be balanced, CD4⁺-biased or CD8⁺-biased, and (b) boosting with the MRKAd5gag construct produced in all cases a strongly CD8⁺-biased response. These results suggest that boosting with MRKAd5HIV-1gag construct is able to improve the levels of antigen-specific CD8⁺ T cells.

Table 24. Boosting of DNA/Adjuvant-Primed Rhesus Monkeys with MRKAd5gag Number of SFChullion PBMCs Grp# Grp#

g L	Priming	Boost Mo	Honk#	T=0		۳	Ta4	ı	T=6	T=10	0	T=17	_	T=24		T=28	_	T=30	e
ĺ	T=0, 4, 8 wrks		=	Wedtum	H GEB	Medium	H BEG	Medium		Medium	H Gag	Medturn	۳	Vedium	H DSD	Medium	H 080	Medium	
_	DNA/5 mgs	MFUCAdSgag(E3+) CE	HSH HSH	≸	ΑN	3	33	15	71	J	727	8	┝	_	2	9	88		316
_	282 282		ĕ	•	0	0	5	•	46	0	89	•	75	_	8	(7)	1705	_	755
	(D101)	A	AW3G	5	F	0	8	6	5	n	8	N	68	60	8	2	886	•	382
T		١	1		1														
N	DNAV5mga +	MRKAd5gag(E3+) CX	ပ္	•	▼	-	8	•	Ξ	w	220	4	280	8	232	3	626	6	1345
	CRL1005/45mgs .		ž	4	0	-	<u>=</u>	0	25	•	Ē	ທ	452	0	젊	•	1915	-	6 69
		Α .	di	6	0	-	2	•	Ξ.	4	亞	80	호	ı,	8	=	88	9	241
		5	<u> </u>	ş	ž	0	ਲ	0	88	0	8	16	374	60	251	80	1549	8	134
		₹	AKBB	<u>.</u>	12	4	8	-	119	0	63	0	23	0	316	7	1229	r)	38
		١											1						
60	DNA/5 mgs+	MFPCAd5gag(E3+) AV	₽ 2	₽	7	-	8	ı,	7 7 7 7 7	6	425	9	ā	60	233	18	565	8	\$
	CRIL 1005/7.5 mgs + 0.6 mM BAK		¥	-	0	6	121	-	135	-	8	s	8	-	103	7	1384	2	878
		3	C858		9	0	9	6	119	•	274	9	282	-	88	0	92	-	828
		8	MS.	4	ຕ	•	8	-	6	0	139	0	<u>\$</u>	-	8	9	3	-	949
		_{បី}	020	-	•	0	136	•	316	-	8	10	83	-	759	•	278	4	1831
ŀ				1	1							1	1	1	1				
4	none	None 980	980201	P 7	0	0	۰	_	0	0	0	0	_	_	01	6	0	0	0
	and an Makin			ĺ													I		۱

EXAMPLE 29

Construction of gagpol fusion for MRKAd5gagpol fusion constructs

The open reading frames for the codon-optimized HIV-1 gag gene was fused directly to the open reading frame of the IA pol gene (consisting of RT, RNAseH and integrase domains) by stepwise PCR. Because the gene (SEQ ID NO: 38) does not include the protease gene and the frameshift sequence, it encodes a single polypeptide of the combined size of p55, RT, RNAse H and integrase (1350 amino acids; SEQ ID NO: 39).

5

10

15

20

25

30

35

The fragment that extends from the BstEII site within the gag gene to the last non-stop codon was ligated via PCR to a fragment that extends from the start codon of the IApol to a unique BamHI site. This fragment was digested with BstEII and BamHI. Construction of gag-IApol fusion was achieved via three-fragment ligation involving the PstI-BstEII gag digestion fragment, the BstEII/BamHI digested PCR product and long PstI/BamHI V1R-FLpol backbone fragment.

The MRKAd5-gagpol adenovirus vector was constructed using the BglII fragment of the V1R-gagpol containing the entire ORF of gag-IApol fusion gene.

EXAMPLE 30

Immunogenicity Studies in Non-Human Primates

Cohorts of three (3) macaques were immunized with 10e8 or 10e10 viral particles (vp) of one of the following MRKAd5 HIV-1 vaccines: (1) MRKAd5gag; (2) MRKAd5pol; (3) MRKAd5nef; (4) a mixture containing equal amounts of MRKAd5gag, MRKAd5pol, and MRKAd5nef, or (5) a mixture of equal amounts of MRKAd5gagpol and MRKAd5nef. The vaccines were administered at weeks 0 and 4.

The T cell responses against each of the HIV-1 antigens were assayed by IFN-gamma ELISpot assay using pools of 20-aa peptides that encompass the entire protein sequence of each antigen. The results (Table 25) are expressed as the number of spot-forming cells (sfc) per million peripheral blood mononuclear cells (PBMC) that respond to each of the peptide pools.

Results indicate the following observations: (1) each of the single gene constructs (MRKAd5gag, MRKAd5pol, or MRKAd5nef) is able to elicit high levels of antigen-specific T cells in monkeys; (2) the single-gene MRKAd5 constructs can be mixed as a multi-cocktail formulation capable of eliciting very broad T cell responses against gag, pol, and nef; (3) the MRKAd5 vector expressing the fusion

protein of gag plus IA pol is capable of inducing strong T cell responses to both gag and pol.

Table 25. Evaluation of Mixtures of MRKAd5 vectors expressing humanized HIV-1 gag, nol. gagnol, nef in rhesus macaques

5

10

Grp#	Vaccine	Monk #			T=6 wks		
·	T=0, 4 wks	j i	Mock	Gag H	Pol - 1	Pol - 2	Nef
1	MRKAd5 gag	CB9V	0	15	•	-	-
	10^10 vp	CD19	ο.	374	-	-	-
	·	109H	1	843	•	•	-
2	MRKAd5 gag	99D130	1	948		-	-
	10^8 vp	W277	16	324	-	-	-
ľ		143H	4	595	-	-	•
3	MRKAd5 pol	CC1X	4	-	46	256	-
	10^10 vp	AW3W	3	1 -	463	550	-
		AV43	6	-	95	1333	-
4	MRKAd5 pol	AW38	1	-	19	30	-
į	10^8 vp	CC8K	0	-	50	995	-
		CC21	1	-	33	· 436	-
5	MRKAd5 nef	076Q	9		-	-	1204
6	10^10 vp	091Q	4	- ,	-	-	85
		083Q	0	-	-	•	176
6	MRKAd5 nef	00C029	1		-	-	114
	10^8 vp	98D022	6	-	-	-	170
		98D160	3	-	-	•	198
7	MRKAd5gag+MRKAd5pol+MRKAd5nef	99D251	3	206	15	193	120
	10^10 vp each	05H	3	135	21	9	638
į		00C016	3	26	4	51	23
8	MRKAd5gag+MRKAd5pol+MRKAd5nef	99D215	1	171	18	193	240
	10^8 vp each	81H	5	73	6	14	243
		12H	8	1140	115	811	719
9	MRKAd5gagpol +MRKAd5 nef	99D211	0	83	56	838	725
	10^10 vp each	22H	4	385	119	1194	1915
		61H	4	343	11	765	853
10	MRKAd5gagpol +MRKAd5 nef	34H	3	78	19	5	75
	10^8 vp each	48H	1	65	105	46	43
		70H	5	158	15	220	191

Indicated are numbers of spot-forming cells per million PBMCS against the peptide pools. Mock, no peptides; gag H, fifty 20-aa peptides encompassing p55 sequence; pol-1, 20-aa peptides representing N-terminal half of IA pol; pol-2, 20-aa peptides representing the carboxy-terminal half of IA pol; nef, 20-aa peptides encompassing the entire wild-type nef sequence. Responses to the antigens prior to the first immunization did not exceed 40 sfc/10^6 PBMC.

WHAT IS CLAIMED IS

10

A recombinant adenoviral vaccine vector at least partially deleted in
 E1 and devoid of E1 activity, comprising:

- a) an adenovirus cis-acting packaging region corresponding to from about base pair 1 to between from about base pair 400 to about base pair 458 of a wildtype adenovirus genome; and
- b) a gene encoding an HIV protein or immunologically relevant modification thereof.
- A vector in accordance with claim 1 comprising a packaging region corresponding to from about base pair 1 to about base pair 450 of a wildtype adenovirus genome.
- 3. A vector in accordance with claim 1 further comprising nucleotides
 15 corresponding to between from about base pair 3511 to about 3524 to about base pair
 5798 of a wildtype adenovirus genome.
 - 4. A vector in accordance with claim 3 comprising base pairs corresponding to 1-450 and 3511-5798 of a wildtype adenovirus genome.
- 5. A vector in accordance with claim 4 which is deleted of base pairs20 451-3510.
 - 6. A vector in accordance with claim 1 which is at least partially deleted in E3.
 - 7. A vector in accordance with claim 6 wherein the E3 deleted region is from base pairs 28,133-30,818.

8. A vector in accordance with claim 1 wherein the gene encoding the HIV protein or modification thereof comprises codons optimized for expression in a human.

- 9. A vector in accordance with claim 1 wherein the vector comprises a gene expression cassette comprising:
 - a) a nucleic acid encoding a protein;

- b) a heterologous promoter operatively linked to the nucleic acid encoding the protein; and
 - (c) a transcription termination sequence.
- 10. A vector in accordance with claim 9 wherein the gene expression cassette is inserted into the E1 region.
 - 11. An adenoviral vector in accordance with claim 9 wherein the gene expression cassette is in an E1 parallel orientation
- 12. An adenoviral vector in accordance with claim 9 wherein the geneexpression cassette is in an E1 antiparallel orientation.
 - 13. An adenoviral vector in accordance with claim 9 wherein the promoter is a cytomegalovirus promoter devoid of intronic sequences.
 - 14. An adenoviral vector in accordance with claim 13 wherein the promoter is an immediate early human cytomegalovirus promoter.
- 20 15. An adenoviral vector in accordance with claim 9 wherein the promoter is a murine cytomegalovirus promoter.
 - 16. An adenoviral vector in accordance with claim 9 wherein the transcription termination sequence is a bovine growth hormone polyadenylation and transcription termination sequence.

17. An adenoviral vector in accordance with claim 9 wherein the transcription termination sequence is a synthetic polyadenylation signal (SPA).

- 18. A cell comprising the adenoviral vector of claim 1.
- 19. Recombinant, replication-defective adenovirus particles harvested
 and purified subsequent to transfection of the adenoviral vector of claim 1 into a cell
 line which expresses adenovirus E1 protein at complementing levels.
 - 20. An HIV vaccine composition comprising purified adenovirus particles of claim 19.
- 21. An HIV vaccine composition of claim 20 which comprises aphysiologically acceptable carrier.
 - 22. A method of producing recombinant, replication defective adenovirus particles containing the adenoviral genome of the adenoviral vector of claim 1 which comprises introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and harvesting the resultant recombinant, replication-defective adenovirus.
 - 23. A method according to claim 22 wherein the cell is a PER.C6[®] cell.

15

- 24. A method of generating a cellular-mediated immune response against HIV in an individual comprising administering to the individual a vaccine of claim 21.
- 25. A method according to claim 24 which further comprises administration to the individual a DNA plasmid vaccine, optionally administered with a biologically effective adjuvant, protein or other agent capable of increasing the immune response.

26. A method according to claim 25 wherein the DNA plasmid vaccine is administered to the individual prior to administration of an adenovirus vaccine.

- 27. A method according to claim 24 wherein the adenovirus vaccine is
 5 preceded by an adenovirus vaccine of a different serotype.
 - 28. A method according to claim 24 which comprises administering and readministering the adenovirus vaccine vector to the individual.
 - 29. An adenoviral vector in accordance with claim 1 wherein the HIV protein is HIV gag or an immunologically relevant modification thereof.
 - 30. An adenoviral vector in accordance with claim 9 wherein the gene expression cassette comprises an open reading frame encoding an HIV gag protein or immunologically relevant modification thereof.
 - 31. A recombinant adenoviral vaccine vector at least partially deleted in E1 and devoid of E1 activity, comprising:
- a) an adenovirus cis-acting packaging region corresponding to from about base pair 1 to about base pair 450 and from about 3511 to about 5798 of a wildtype adenovirus genome, and deleted for base pairs corresponding to from about base pair 451 to from about base pair 3510 of a wildtype adenovirus genome; and
 - b) a gene expression cassette comprising
 - i) SEQ ID NO: 29;
 - ii) a heterologous promoter operatively linked to i); and
 - iii) a transcription termination sequence.

32. An adenoviral vector in accordance with claim 31 wherein the gene expression cassette is in an E1 parallel orientation.

- 33 An adenoviral vector in accordance with claim 31 wherein the gene expression cassette is in an E1 antiparallel orientation.
- 34. An adenoviral vector in accordance with claim 31 wherein the promoter is a cytomegalovirus promoter devoid of intronic sequences.

5

15

- 35. An adenoviral vector in accordance with claim 31 wherein the transcription termination sequence is a bovine growth hormone polyadenylation and transcription termination sequence.
- 36. An adenoviral vector in accordance with claim 31 which is at least partially deleted in E3.
 - 37. A cell comprising the adenoviral vector of claim 30.
 - 38. Recombinant, replication-defective adenovirus particles harvested and purified subsequent to transfection of the adenoviral vector of claim 30 into a cell line which expresses adenovirus E1 protein at complementing levels.
 - 39. An HTV vaccine composition comprising purified adenovirus particles of claim 38.
 - 40. An HIV vaccine composition of claim 39 which comprises a physiologically acceptable carrier.
 - 41. A method of producing recombinant, replication defective adenovirus particles containing the adenoviral genome of the adenoviral vector of claim 30 which comprises introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and harvesting the resultant recombinant, replication-defective adenovirus.

42. A method according to claim 41 wherein the cell is a PER.C6® cell.

43. A method of generating a cellular-mediated immune response against HIV in an individual comprising administering to the individual a vaccine of claim 21.

- 44. A method according to claim 43 which further comprises administration to the individual a DNA plasmid vaccine, optionally administered with a biologically effective adjuvant, protein or other agent capable of increasing the immune response.
- 45. A method according to claim 44 wherein the DNA plasmid vaccine is administered to the individual prior to administration of an adenovirus vaccine.
 - 46. A method according to claim 43 wherein the adenovirus vaccine is preceded by an adenovirus vaccine of a different serotype.
- 47. A method according to claim 43 which comprises administering and readministering the adenovirus vaccine vector to the individual.
 - 48. An adenoviral vector in accordance with claim 1 wherein the HIV protein is HIV pol or an immunologically relevant modification thereof.
- 49. An adenoviral vector in accordance with claim 9 wherein the gene
 20 expression cassette comprises an open reading frame encoding an HIV pol protein or immunologically relevant modification thereof.
 - 50. A recombinant adenoviral vaccine vector at least partially deleted in E1 and devoid of E1 activity, comprising:

a) an adenovirus *cis*-acting packaging region corresponding to from about base pair 1 to about base pair 450 and from about 3511 to about 5798 of a wildtype adenovirus genome, and deleted for base pairs corresponding to from about base pair 451 to from about base pair 3510 of a wildtype adenovirus genome; and

5

- b) a gene expression cassette comprising
 - a nucleotide sequence selected the group consisting of SEQ ID NO: 1, SEQ ID NO: 5 and SEQ ID NO: 7;
 - ii) a heterologous promoter operatively linked to i); and
 - iii) a transcription termination sequence.

10

- 51. An adenoviral vector in accordance with claim 50 wherein the gene expression cassette is in an E1 parallel orientation.
- 52. An adenoviral vector in accordance with claim 50 wherein the gene expression cassette is in an E1 antiparallel orientation.

15

- 53. An adenoviral vector in accordance with claim 50 wherein the promoter is a cytomegalovirus promoter devoid of intronic sequences.
- 54. An adenoviral vector in accordance with claim 50 wherein the transcription termination sequence is a bovine growth hormone polyadenylation and transcription termination sequence.

- 55. An adenoviral vector in accordance with claim 50 which is at least partially deleted in E3.
 - 56. A cell comprising the adenoviral vector of claim 49.

57. Recombinant, replication-defective adenovirus particles harvested and purified subsequent to transfection of the adenoviral vector of claim 49 into a cell line which expresses adenovirus E1 protein at complementing levels.

- 58. An HTV vaccine composition comprising purified adenovirusparticles of claim 57.
 - 59. An HIV vaccine composition of claim 58 which comprises a physiologically acceptable carrier.
 - 60. A method of producing recombinant, replication defective adenovirus particles containing the adenoviral genome of the adenoviral vector of claim 49 which comprises introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and harvesting the resultant recombinant, replication-defective adenovirus.

10

- 61. A method according to claim 60 wherein the cell is a PER.C6® cell.
- 62. A method of generating a cellular-mediated immune response against HIV in an individual comprising administering to the individual a vaccine of claim 59.
 - 63. A method according to claim 62 which further comprises administration to the individual a DNA plasmid vaccine, optionally administered with a biologically effective adjuvant, protein or other agent capable of increasing the immune response.
 - 64. A method according to claim 63 wherein the DNA plasmid vaccine is administered to the individual prior to administration of an adenovirus vaccine.

65. A method according to claim 62 wherein the adenovirus vaccine is preceded by an adenovirus vaccine of a different serotype.

- 66. A method according to claim 62 which comprises administering and readministering the adenovirus vaccine vector to the individual.
- 67. An adenoviral vector in accordance with claim 1 wherein the HIV protein is HIV nef or an immunologically relevant modification thereof.
 - 68. An adenoviral vector in accordance with claim 9 wherein the gene expression cassette comprises an open reading frame encoding an HIV nef protein or immunologically relevant modification thereof.
 - 69. A recombinant adenoviral vaccine vector at least partially deleted in E1 and devoid of E1 activity, comprising:
 - a) an adenovirus *cis*-acting packaging region corresponding to from about base pair 1 to about base pair 450 and from about 3511 to about 5798 of a wildtype adenovirus genome, and deleted for base pairs corresponding to from about base pair 451 to from about base pair 3510 of a wildtype adenovirus genome; and
 - b) a gene expression cassette comprising
 - a nucleotide sequence selected the group consisting of SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13 and SEQ ID NO: 15;
 - ii) a heterologous promoter operatively linked to i); and
 - iii) a transcription termination sequence.
 - 70. An adenoviral vector in accordance with claim 69 wherein the gene expression cassette is in an E1 parallel orientation.

15

5

71. An adenoviral vector in accordance with claim 69 wherein the gene expression cassette is in an E1 antiparallel orientation.

- 72. An adenoviral vector in accordance with claim 69 wherein the promoter is a cytomegalovirus promoter devoid of intronic sequences.
- 73. An adenoviral vector in accordance with claim 69 wherein the transcription termination sequence is a bovine growth hormone polyadenylation and transcription termination sequence.
 - 74. An adenoviral vector in accordance with claim 69 which is at least partially deleted in E3.
 - 75. A cell comprising the adenoviral vector of claim 68.

10

15

- 76. Recombinant, replication-defective adenovirus particles harvested and purified subsequent to transfection of the adenoviral vector of claim 68 into a cell line which expresses adenovirus E1 protein at complementing levels.
- 77. An HIV vaccine composition comprising purified adenovirus particles of claim 76.
 - 78. An HIV vaccine composition of claim 77 which comprises a physiologically acceptable carrier.
 - 79. A method of producing recombinant, replication defective adenovirus particles containing the adenoviral genome of the adenoviral vector of claim 68 which comprises introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and harvesting the resultant recombinant, replication-defective adenovirus.
 - 80. A method according to claim 79 wherein the cell is a PER.C6® cell.

81. A method of generating a cellular-mediated immune response against HIV in an individual comprising administering to the individual a vaccine of claim 78.

- 82. A method according to claim 81 which further comprises
 administration to the individual a DNA plasmid vaccine, optionally administered with a biologically effective adjuvant, protein or other agent capable of increasing the immune response.
- 83. A method according to claim 82 wherein the DNA plasmid vaccine is administered to the individual prior to administration of an adenovirus vaccine.
 - 84. A method according to claim 81 wherein the adenovirus vaccine is preceded by an adenovirus vaccine of a different serotype.
 - 85. A method according to claim 81 which comprises administering and readministering the adenovirus vaccine vector to the individual.

15

- 86. A multivalent adenovirus vaccine composition comprising recombinant, replication-defective adenovirus particles, wherein the adenovirus particles are harvested and purified from a cell line expressing adenovirus E1 protein, and wherein the particles are harvested subsequent to transfection of the cells with an adenoviral vector or vectors in accordance with claim 9; said vector(s) comprising a gene expression cassette or cassettes comprising nucleotide sequences encoding HIV proteins selected from the group consisting of:
 - a) gag, pol, and nef, expressed independently from three individual vectors;

5

)

5

)

b) gag, pol, and nef, expressed independently from one vector with the encoding nucleic acid sequences operatively linked to distinct promoters and transcription termination sequences; c) gag, pol, and nef, expressed via two vectors, one expressing a polnef fusion, and another expressing gag; d) gag, pol, and nef, expressed via two vectors, one expressing a gagpol fusion and another expressing nef; e) gag, pol and nef, expressed via two vectors, one expressing a nefgag fusion and another expressing pol; f) gag, pol, and nef, expressed via one vector expressing a gag-pol-1(nef fusion; g) gag and pol, expressed independently from two individual vectors; h) gag and pol, expressed independently from one vector with the encoding nucleic acid sequences operatively linked to distinct promoters and transcription termination sequences; 15 i) pol and nef, expressed independently from two individual vectors; j) pol and nef, expressed independently from one vector with the encoding nucleic acid sequences operatively linked to distinct promoters and transcription termination sequences; k) nef and gag, expressed independently from two individual vectors; 20 1) nef and gag, expressed independently from one vector with the encoding nucleic acid sequences operatively linked to distinct

promoters and transcription termination sequences;

m) gag and pol, expressed via one vector expressing a gag-pol fusion;

n) pol and nef, expressed via one vector expressing a pol-nef fusion; and

- o) nef and gag, expressed via one vector expressing a nef-gag fusion.
- 87. A multivalent adenovirus vaccine composition in accordance with claim 86 wherein the gag-pol fusion consists of SEQ ID NO: 39.

- 88. A multivalent adenovirus vaccine composition in accordance with claim 86 wherein the fused sequences have the encoding nucleic acid sequences operatively linked to distinct promoters and transcription termination sequences.
- 89. A multivalent adenovirus vaccine composition in accordance with
 10 claim 86 wherein the fused sequences have the encoding nucleic acid sequences
 operatively linked to a single promoter; and the encoding nucleic acid sequences
 operatively linked by an internal ribosome entry sequence ("IRES").

Original Adenovector Construct:

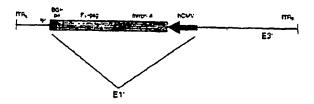


Figure 1: Original HIV-1 gag adenovector.

Sequence of the open reading frame for FL-gag (human codon optimized)

atgggtgctagggcttctgtgctgtctggtggtgagctggacaagtgggagaagatcaggctgaggcctggtgg caagaagaagtacaagctaaagcacattgtgtgggcctccagggagctggagaggtttgctgtgaaccctggc agctgaggtccctgtacaacacagtggctaccctgtactgtgtgcaccagaagattgatgtgaaggacaccaag gaggccctggagaagattgaggaggagcagaacaagtccaagaagaaggcccagcaggctgctgctggc acaggcaactccagccaggtgtcccagaactaccccattgtgcagaacctccagggccagatggtgcaccag gccatctcccccggaccctgaatgcctgggtgaaggtggtggaggagaaggccttctcccctgaggtgatccc catgitetetgecetgictgagggtgccacccccaggacctgaacaccatgctgaacacagtgggggggccatc aggetgecatgeagatgetgaaggagaceatcaatgaggaggetgetgagtgggacaggetgeatcetgtge acgctggccccattgcccccggccagatgagggagcccagggggctctgacattgctggcaccacctccaccct ccaggagcagattggctggatgaccaaccaccccccatccctgtgggggaaatctacaagaggtggatcat cccttcagggactatgtggacaggttctacaagaccctgagggctgagcaggcctcccaggaggtgaagaact ggatgacagagaccctgctggtgcagaatgccaaccctgactgcaagaccatcctgaaggccctgggccctg ctgccaccctggaggagatgatgacagcctgccagggggtggggggccctggtcacaaggccagggtgctg gctgaggccatgtcccaggtgaccaactccgccaccatcatgatgcagagggcaacttcaggaaccagag gaagacagtgaagtgcttcaactgtggcaaggtgggccacattgccaagaactgtagggcccccaggaaga agggetgetggaagtgtggcaaggaggccaccagatgaaggactgcaatgagaggcaaggccaacttcctg ggcaaaatctggccctcccacaagggcaggcctggcaacttcctccagtccaggcctgagcccacagcccct agetglacecectggeeteetgaggteetgtttggeaacgaceceteeteecagtaaaataaageeegggea gat (SEQ ID NO: 29)

Figure 2

Old Transgene:



New Transgenes:

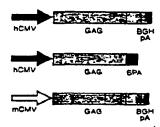


Figure 3: Diagrammatic representation of the original HIV-1 gag transgene and the series of new transgene constructions.

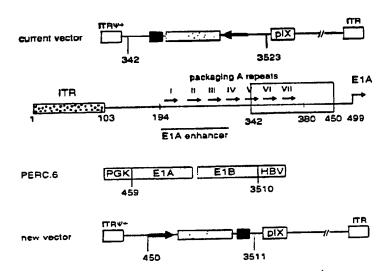


Figure 4: Modifications made to the current adenovector backbone in the generation of the new vector.

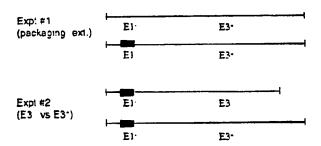


Figure 5: Virus mixing experiments to determine the effects of the addition made to the packaging signal region (Expt #1) and analysis of the effects of the E3 gene on viral growth (Expt. #2). The red bars denote the region of modifications made to the E1 deletion.

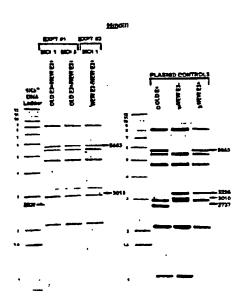


Figure 6: Autoradiograph of viral DNA analysis following viral mixing experiments (expts. #1 and #2) as detailed in the text.

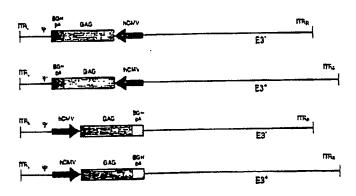


Figure 7A: hCMV-FLgag-bGHpA adenovectors constructed within the "MRK" backbone. E1 parallel and E1 antiparallel transgene orientation within the E3- and E3+ backbones were constructed.

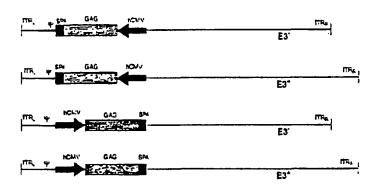


Figure 7B: hCMV-FLgag-SPA adenovectors constructed within the "MRK" backbone. E1 parallel and E1 antiparallel transgene orientation within the E3- and E3+ backbones were constructed.

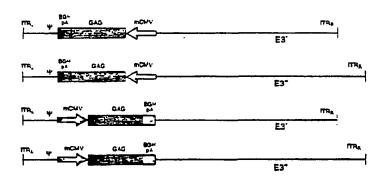


Figure 7C: mCMV-FLgag-bGHpA adenovectors constructed within the "MRK" backbone. E1 parallel and E1 antiparallel transgene orientation within the E3- and E3+ backbones were constructed.

Plasmid mixing expt: (orientation)

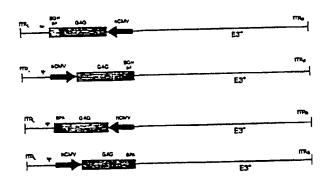


Figure 8A: Effect of transgene orientation

Plasmid Mixing expt: (poly A signal)

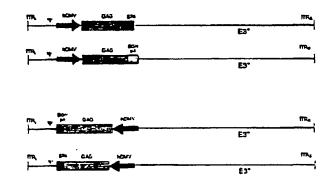


Figure 8B: Effect of polyadenylation signal

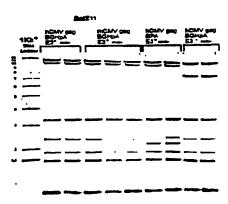


Figure 9: Viral DNA from the four Adgag candidates at P5, following BsfE11 digestion.

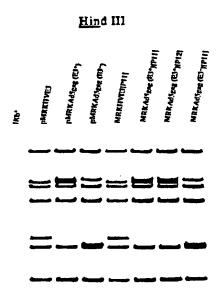


Figure 10: Viral DNA analysis of passage 11 and/or 12 of MRKHVE3, MRKAd5gag and MRKAd5gag(E3-).

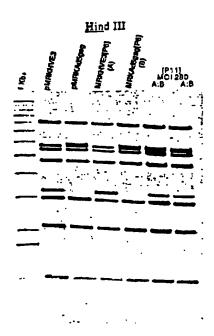


Figure 11: Viral DNA analysis (*Hind*III digestion) of passage 6 MRKHVE3 and MRKAd5gag used to initiate the viral competition study. Last two lanes are passage 11 analysis of duplicate passages of the competition study (each virus at MOI 280 vp).

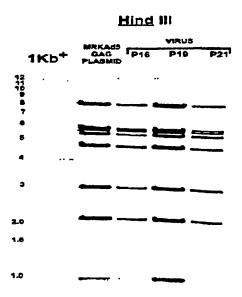
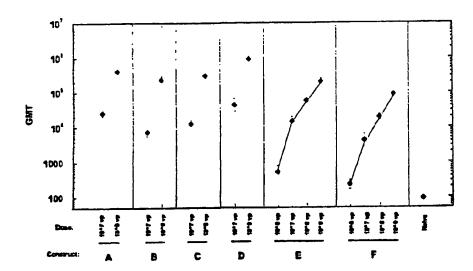


Figure 12: Viral DNA analysis by *HindIII* digestion on high passage numbers for MRKAd5gag in serum containing media with collections made at specified times. The first lane shows the 1 Kb DNA size marker. The other lanes represent pre-plasmid control (digested with Pac1 and *HindIII*), and MRKAd5gag virus continually passaged to P16, P19 and P21(serum containing media).

Figure . Serum anti-p24 Levels at 3 Wks post i.m. immunization of balb'c mice (n=10) with Varying Doses of Several Adgag constructs: (A) MRKAd5gag (through passage 5): (B) MRKAd5 E3' hCMV-FLgag-bGHpA; (C) MRKAd5 E3' hCMV-FLgag-SPA; (D) MRKAd5 E3' mCMV-FLgag-bGHpA; (D) research Lot (293 cell-derived) of Ad5HIV-lgag; and (F) clinical lot (Ad5gagFN0001) of Ad5HIV-lgag. Reported are the geometric mean titers (GMT) for each cohort.



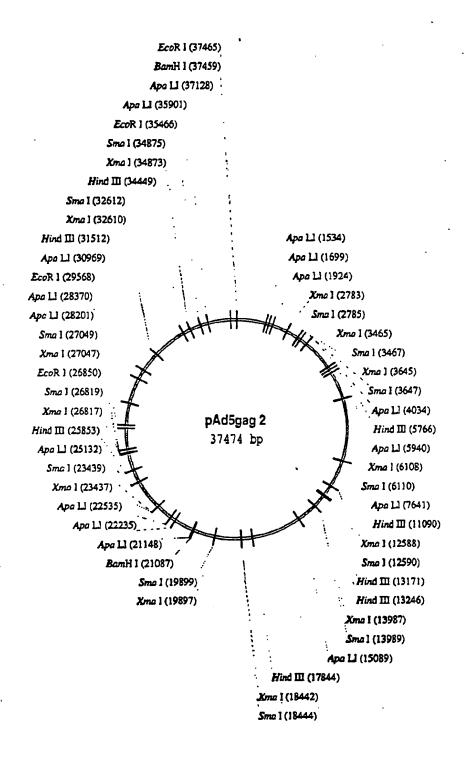


Figure 14

PHYSTERACTI GOCHEGORDE GIVEGINALIGE ANCHETGEN CCCCARCICCO CACCUTTGI''	GROGENAMO TOACOTITIT GETETITIS: CACCOTITIC ACTOCANAA CCACACCO:	GCCATITITICG	-	TACCHTCTAT		TCACCTCAAT	ACTICCCACT ACATCAAGTG TATCATATY; TGAACCGTCA TGTAGTTCAC ATAGTATAC:	ATGGGACTTT CCTACTTGGC AGTACATCTA TACCCTGAAA GGATGAACCG TCATGTAGAT	GACTICACCOG GATTTCCAAG TCTCCACCC CTGAGTGCCC CTAAAGGTTC AGAGTTKAG	CCTTTACCC	CTGTTTTGAC CTCCATAGNA GNCACCCGGGA GNCAAAACTG GAGGTATCTT CTGTGGCCCCT	CATGGGTACT AGGGCTTCTG TGCTGTGTGGGGTACCGGGAAGCC	ATTOTOTOO CETECARON GETRINARIAGO TAACACACCE GGAGGTCCET CGACCTETIC	CCCTOCADAC AGGENTING CAGETGAGET COGACGETTO TCCGAGACT CTCGACTETA	ATTONGONGO TAACTCCTCC	ATTOTIGENGA ACCTICCANGGO CCAGATUGTIC TAACACGTCT TGGAGGTCCC GGTCTACCAC
CCACCACCTC	ACCIOCOTA TOCHOCOTA	CCCATACCGA	אסרומכנימרס זרככימפניסכים	בניסכנססנסכ סייניסכנכסינס	CTACTTATTA GATCAATAAT	GCCCAACGAC	TANACTIGCCC	ACATGACCTT TGTACTGGAA	ATACCCANA TATCCCCANA	AACAACTCCG TTCTTCAGGC	GCCATCCACG CGGTMAGTGC RgH	TCACATCTAC ACTCTAGATG	CCATTICCTC	CHECAGECET	CCANDANGC	CHICATCOCC
ATCATAATCA TACTATTACT	AACACATGTA TTGTGTACAT	CATTFRANCT:	ATATTTGTCT TATAMGAGA	TATTATTATA ATAATAATAT	TCATTATTCA ACTAATAACT	CHGGCHGACC	CATANATIACCE CATANATIACC	TATGCCCAGT ATACGCCTCA	ATTACTACTATES TACTACTACACA	AAAATETEGET TTTTACAGCA	GCCTCTCTAGAC	CACCATTACTC	ACAAGTACAA TCTTCATGTT	CCTGGGCCAG GGACCCGGTC		AGGERATECEA
TGANGTCAAT ACTIX YASTIA	ACTITITIVACIAS TCACACCGCC	GCTACATOTA CCTACAACAT	ATACTIC CITA TATES COCAT	CANCICICANA	ATCTTCACAT TACAACTGTA	ANTEGECICAE TTACCGGGCG	AATCCCACCT	CCCCTGCCAT	CAGTACATCA GTCATGTAGT	CCCTGAAAGG	CCGTCAGATC	CCGATTCCCC	CCACCGTTCT	GCAGGCAGAT	GAAGATTGAT CTTCTAAGTA	אארידרכאמרנר דייאמידרקמ
TATTTTKEAT	TCATCTTCCA	CCAAAATCCG	TYTICTTACTC ACACAATGAG	CCCGGTCAAA	CATTACCGCC GTAATGGCGG	ACTTACGGTA TGAATGCCAT	CATTGACGING	CATTTACCOS	CCCCANANCE	CANANTCAAC	GTTTAGTKAN CANATICACTT	CATTGGAACG	GCTGAGGGGT CGACTCCGGA	TCTGAGGGGT AGACTCCCCA	GTGTCCACCA CACACGTCCT	TGGC NC NGC ACCGTGTCCG
TAAFATACT	CCCCCTTCAC	ATTTECTOR	TCANTANTIT	TTTCCCCCTT ANACRCCCNA	TCATCTCCAA	GCGTTACATA CGCAATGTAT	AGGGACTITIC TCCCTGAAAO	GICANTGACG	CCATCCTCAT GGTACCACTA	GTTTTGGCAC CAAAACCGTG	AGCAGAGCTC TCGTCTCGAG	GOGNACAGTA	AGAAGATCAG	OCTOGAGACC CGACCTCTGG	ACCCTGTACT TGGGACATGA	ARGETRICTRIC TECEBATGACG
ACATCATCAA TOTACTA	GINGTAGICIT	OGAAGTGACA	AAGTGAAATC TTCACTTTAG	TCTCAGGIGT	TTATATTGGC AATATAACCG	ATCCACTTCC TACCTCAAGG	TAACOCCAAT	CCCTATTGAC	ATCGCTATTA	ATCCCTCAAA	GGECTATATA CCAGATATAT	CHCCGCGGCC	CHSTICACC	ACCCTOOCCT TOOGACCOGA	CACAGTOGCT	AAGGCCCAGC TICCCGGGTCG
ANCANTTANT S	CCCCCACTG (GANTAAGAGG	CAGGIGITIT	ATATGTACAT	TAGCCCATAT	GTTCCCATAG CAAGGGTATC	CAAGTACGCC	CGTATTAGTC	ATTGACGICA	TACCOTCCCA	CCGATCCAGC	TYSCTCAGCTC ACCACTCGAC	TTTOCTGTGA	CCCTGTACAA	GTCCAAGAAG
-	101	201	301	401	501	601	101	108	901	1001	1101	1201	1301	1401	1501	1601

Figure ISA

PMRKA/15qaq MER682

1701	CACCAGGCCA		GACCCTCAAT		A:cn::cm:::/A	ALITHTINYIA CHANGAAGGCC	TICTICACTO	AGGTGATCCC	CATGETCTCT	GCCCTGTCTG
	6166171661	-	_		11.W.T.M.T.T		ANCHICKACAC	TCCACTAGGG	GTACAAGAGA	בנאאימכמכמכ
1801	TECCACGGTG	CCCCCAGGAC	GACTTREPTER	ACCIAL TITIONS	MCMTTTTC	CTATE ACEDICAL	CCATCCACAT	CGACTTCCTC	ACCATCAATG	AGGAGGCTG-
1901	TEAGTRAGEAC					ACATICACICA	CATCLACTORY	Theresacames	CHASTACCAC	
!	ACTCACCCTG	TCCGACGTAG			נושאאמאורנות	TYTACTORCY	CONTRACTOR	AGACTIGTAAC	GACCGTGGTG	GAGGTGGGAVS
2001	CAGGAGCAGA	TICCCICCAT	GACT: MATING	CCCCCCATIC	VIXIONALIA	ANTICTACAAG	AKKTICKCATEA	recrement	GANCAAGATT	GTGACTATET
	Greener	AACCGACCTA	CTGGTTK;TTG	COCCACCTAGG	מאנאנגנכנד	TTAGATGETE	TCCACCTAGT	AGGACCCGGA	CTTGTTCTAA	CACTCCTACA
2101	ACTOCOCOCAC	CICCAICCIG	GACATCANCE	ACKERICCEAN	ואישנינינדונ	AGGACTATG	TGGACAGGTT	CTACAAGACC	CTCAGGGCTG	AGCANGCCT.
	TOAGGGGGTG	CAGGTAGGAC	CTGTAGTCCC	recedengerr	CCTCCCCCAAG	TCCCTCATAC	ACCTGTCCAA	GATGTICTOG	GACTCCCGAC	TCGTCCGGA:
2201	CCAGGAGGTG	-				CAGAATGCCA ACCCTGACTG	CANGACCATC	CTGAAGGCCC	TGGGCCCTGC	POCCACCETO
	OGTECTECAC	THETTOMECT	ACTOTOTOTO	GGACGACCAC		CTCTTACCCT TCGCACTCAC	CTTCTGGTAG	GACTTCCGGG	ACCCOGGRCG	ACGGTGGGW
2301	DAGGAGATGA					CAGGGTGCTG	GCTGAGGCCA	TOTCCCAGGT	GACCAACTCC	GCCACCATC,
,	CICCICINCI				CMSTRITCCG	CAGINETICES GIECEACTAC	CGACTCCGGT	ACAGGGTCCA	CTGGTTGAGG	COGTOGTACT
2401	TCATCCACAG				מאת וומכידוכ	CAACTGCTTC AACTGTGGCA	ACCTCCCCA		MACTIGITACIO	CCCCCCM30AV.
	ACTACOTOR	CCCGTTGAAG	rccrrccrc	CCTTCTGTCA	CTTCACGAAG	THINCACCOST	TCCACCCAGT	GTAACGGTTC	TTGACATCCC	GOCHOTICET "
2501	GANGGOCTGC		GCANGGAGG	CCACCAGATG	AAGGACTGCA	AAGGACTGCA ATGAGAGGCA	GCCAACITIC	CTCCCCAAA	TOTOGOCOTIC	CCNCNACIOCH:
	CTTCCCGACG	ACCTICACAC	COTTCCTICC	GGTGGTCTAC		PICCIGACGE PACTOTOCOF	CCCCTTCAAG	GACCCONTIT	AGACCGGGAG GCTCTTCCCX:	GGTCTTCCCC
2601	AGGCCTGGCA	_	GTCCAGGCCT	GAGCCCACAG		CCCCTCCCGA GGAGTCCTTC	AGGTTTGGCG	ACCACANGAC	CACCCCCAGC CACIAAGCAC	CACIANGCAR
	TCCCGACCGT	TGAAGGAGGT	CAGGICCGGA	CTCCCCTCTC	CCCCACCACCT	CCTCAGGAAG	TCCAAACCCC	recremeno	מדסססכקרכם הדכודוכבדכי	GICT-ICCTC:
									-	3/101 Avvone
2701	AGCCCATTGA	CAAGGAGCTG	TACCCCCTRIG	CCTCCCTGAG	GRECETATIFF	CACANTINCE	CCTCCTCCCA	GTANNITAAA	OCCUBARCAG AICTUCTOR	ATCTOCTOTO:
	TCGGGTAACT	GITCCICGAC	ATCCCCCCACC	GENERALITY	CACCCACAAA	CCCTTCCTIGG	CCACCACCCT	CATITITATIT	COCOCCCORC	TAGACGACA
2801	CCTTCTAGT	GCCAGCCATC	Temerringe	CCCTCCCCCGG	TOCCITICON	GACCETGGAA	GGTGCCACTC	CCACTGRCCT	TTCCTAATAA	ANTGAGGAAN
	GGAAGATCAA	COGREGOTAG	ACANCAAACG	CCCACCCCCC	ACCIGAAGGAA	CTGGGACCTT	CCACGGTGAG	GCTCACAGGA	AAGGATTATT	TTACTCCTTT
,										Spti
2901	THOCARCOCA	THETCHOAGT		ADDITION OF THE CHAINCHANG RAGINGORY RECONSERVA	CONTRACTOR	CHICCOLKEACA	CCAACCATTA CCATTCCCAA	CCATTICCCAA	GACAATAGCA	GCCATCACTCG
	ACCTAGCGT	AACAGACTCA	-	TCCACAGTAA GATAAGACCC	CCCACCCCAC	cccercenst	CGTTCCCCCT	CCTAACCCTT	CTGTTATCGT	CCCTACGACC
			P. A.	Asd						
3001	GGATGCGGTG	GOCICIATED	CCGATCGGCG	GGANGCOGIG GOCICTATOG CCGANCGCG (CCCGIACTIC AAATGIVINX) (XXINXXIIIA AGGGIXAGAA AGAATAIATA AGGIGGGGGG CTITAIGIAGG	AAATCTCTT	CASCELLA TOTAL	AGGETGGGAA	AGAATATATA	AGGTGGGGGT	CTTATGEAGT
	CCTACGCCAC	CCGAGATACC	GGCTAGCCGC	מסכדאה כנה הכמה אוהאכ	TTTACACACC	TITTACACACC COCACCGAAT TYCCACCCTT TCTTATATAT	TYCCACCCTT	TCTTATATAT	TCCACCCCCA	GANTACATCA
									Sphi	
3101	THURATUR		פככפכבעכנפ	THITIGAGCA GCCGCCCCC CCATGAGCAC CAACTCGTTT GATGGAAACCA	CAACTERSTT	GATGUAAACA		THEREACTE ATAPPEACE	ACOCCCATCC CCCCATACCC	CCCCATACAC
	AAACATAGAC		200000000	AMACCICAT COCCOCCAC GOTACICATIC CITICAGIANA CTACITITATE	CTTCAGCAAA	CTACCTTCGF	ANCACTOGAG TATABACTOT	TATABACTOT	TOCOCOTACO COCOTACCO	GOOGTACCC13
3201	COCOCINCOCT		TOTAL TOTAL	CAGAANGNIA NIYYYYTGAG CATNAANAGT CGCCCCGTTC NICCYACAAA CTCTACTACC TRACCTACG AGACCGGIC YGGAACGCGG	ودور در در در دوالد د	TRACCOGRAMA	CTCTACTACC	TTGACCTACG	AGACCCTCTC	TOCHACCCCG
	CCCCACGCA	GTCTTACACT	ACCCGAGGTC	BOCCCAGGCA GTCTTACACT ACCCGAGGTC GTAACTACCA GCGGGGGTACAG ACGGGCGTTT GAGATGATGG	CCCCCCCCCACACC	ACCOCCTT	GAGATGATGG	AACTGGATGC	TCTGGCACAG ACCTTGGGGC	ACCTTGGGG

tique 150

	4	91		15.7						
,,,,	THE PERSON NAMED IN COLUMN NAM	A PER CHARACTER AND A PER	Carrichmy	אייייייייייייייייייייייייייייייייייייי		COGGATTERS A	ACTICACTITIES C	CPTRCTGAG (AACAC:TGCAG
1000				כאמורוארידור: מאויוראורואאר		נאיניריויאתנאני	TGACTGAAAG C		GCCCCAACGT	THETHEREGIE
3401			CATCACAGT	TGACKERTICT	TTTYRECALTAN					ACKTINGTINGTA
))	_		CTACTOTACA	ACTRECOGNGA	AAACCCTCTT	איככיזיאיניאי		_		ובכניאכ אינו יו.
3501			כככדייאאיהיב			TTTAMACAT	MANTAAAAA (CCAGACTICTG	AAACCTAMC	GATCAAGCAA CTAGTTGGI I
			CCCACTTCCG							AGGACGTG.T
3601	CACACARACCA	CAGANATANA	ACCCCAAAAC	CONTROCCION OF STREET	ARRECTION TO THE TANK	GENERALINA				TCCTGCACCA
							Pst1			
1701	ABACTTACT	CHOGATOTIC	AGATACATGG	CCATAAGGC	GPCTCTOGGG	TYX;ACCTAGE				TOTTOTAGAT
5		GACCTACANG			כאליאניאניביו כ	ACCTICATEG	TOCHERCOIC .		ACCCCCCACC	ACAACATICTA
3801		TAGCAGGAGC	GCTOCACCATA	GTGCCTANAA	ANCHULLINGA				TCGTGTAAGE	CTTTACAAN:
· •	CTAGGTCAGC	ATCGICCTCG	CGACUTTICAC	CACGGATTTT	TACATAAAGT		_		ACCACATICA	CAMILETITE
1901	COCHTAGET	COCATOGGTO	CATACCITICS	CATATGAGAT	GCANCTINASA	CHUTATITI			CATATCCCTC	COCCATATIVA
	OCCAATICGA	CCCTACCCAC	OTATOCACCC	CTATACTYTA	CGTA:MCCT	GACATANAA		-	Gratagggag	GCCCCTAMGT
4001	TOTTOTOCAG	AACCACCAGC	ACAGTGTATC	COCTUXACTT	GGGANATHG	TCATGTAGGT			MACTITGGAGA	COCCUTTON
! •	ACAACACGIC	Trocrocres	TETCACATAG	GCCACCTIGAA	CCCTTTANAC	AGTACATOGA	ATCITICCTIT	ACGCACCETIC	TIGNACCICT	GCCCA : AACAC
4101	ACCTCCANGA	THICCATOC	ATTCGTCCAT	AATTATGGGA	ATTACACTORIC	ევტელების	CTASCCGAAG	ATATTICTOG	GATCACTAAC	GICATAGITIG
	TOGAGGITCT	AAAAGGTACG	TANGCAGGTA	TTACTACCGT	TACCOGGTG	נרבטכנעננפ	GACCCGCTTC	TATAAAGALC	CIMEIGNING	CANTAICAN
4201	TOTTICCAGGA	TCAGATCGTC	ATAXXCATT	TTTACAAAGC	CCCCCCCCCCAAG	CHETCHOLD	TCCCCTATAA	TOGITICCATC	COGCCCAGOG	GCGTAGTTA
	ACAAGGTCCT	ACTCTAGCAG		AAATGTTTCG	COCCCGCCTC	CCACGGTCTG	ACCCCATATE	ACCNACCTAG	accocancec	CCCATCAA'n:
A 101	CONTRACTOR T	TRICATTICC	_	CITCAGATOG	CXXCATCATG	TCTACCTGCG	GGGCGATGAA	GAAAACGGTT	TCCCGCCTAG	GGGAGIATICA
	OCACTUTA	AACGTAAAGG		CAAGTCTACC	CCCCTAGTAC	AGATGGACGC	CCCCCTACTT	CTTTTCCCAA	AGGCCCCATC	CCCTCTAGTC
										ANTO
4401	CTCGGAAGAA	AGCAGGITCC	TONOCACCTO	CCACTTACCG	CACCTOGING	CARICTIONING GOOGGENANT CACACCTATT	CACACCTATT	ACCERCATECA	ACTOGTACT	AAGACMXCT):
! •	CACCCTACTT	_	ACTCGTCGAC	GCTYANTYGG	CHECOCCACC	COCCATITA	GTGTGGATAA	TOCCCCACGI	TCACCATCAA	TICICICAR
	Psc									
4501	CAGCTGCCGT	CATCCCTGAG	CAGGGGGGGCC	ACTRICOPTIAN	CCATGTCCCT	GCATGTCCCT GACTCCTCATATG				TELECTIALETA
	GTCGACOCCA	GTACOGACTC	: فيدددددوو	TCAAGCANTT	CGTACAGAGA	CTGAGGGTAC	ANANGOGACT	GGTTTACGCG	GICTICCACE	MACINACIONAL
4601	GCCATAGCAG	-	_				GCATACCTTT	CACCULTICA	CEAMBLAGIT	COMOCCOLC
	CACTATOOTO	ANGANCOTTIC	: CTRCCTITCA	ANANGTICCC						
4701	CCACACCTCG	OTCACCTOCT		-					COCACING	בראי איני דיניי
	GGTGTCGAGC	: CAGTGGACGA	A CATCCCCTAG	ACCTACATICG					מרכנוראורא	
4801	CCAGACGGGC	: CAGGGGTCATG	3 TICTITICCACG		נכוגייוניאיני				CCCAGCTIACG	
	GCTCTGCCCGG	GICCCAGTAC	2 AGAAAGGTKK		המשכבובהבת מפשטמשיבום	CATCAGACCC	AGTOCCACTT	CCCCACGCGA	CECCEGACIAL	ואכיאוירואסיאי

figure 15c

1064	acracactra	AGGCTOGTCC	TOCHOOTICE	CAACECTATIC						GICCAGROCK
	CCACGCGAAC	TCCGACCAGG	ACCIACCACGA	בשמטכנועני	CCCACACACC	היאנדטריבאה	כנינייונינאדכ			CAGGITCHAGA
	7000000T	GOCCEPTING (OCCCAGCTTC	CCCTTVX:Arx	ArascriciacA	じくごりどうじんべい		_		GCGAGAAATA
	AGGCGCCGCA	CCGGGAACCG	CCCCTCGAAC	COCCANCUTOC	TCCGCGCGT	CX TRICRICGTC	ACCITCITICIANA	ACTCCCCCAT	CTCGAACCCG	CGCTCTTTAT
	CCGATTCCGG	GGNOTAGGCA	TCCACGCCGC	AGGCCCCCCCA	GACKETETE	CATTECACGA	CUCCHARTERAG	CTCTGGCCGT		ANACCAGGIT
	GCCTAAGGCC		AGGCGCGGCG	recesser	CTRCCAGAGE	GTAAGGTGCT	כפסוככאכוב	GAGACCGGCA	AGCCCCAGTT TT	TTTGGTCCA:
	Tececontoe	THEFTGATGE	GPPICTTACC	TCTCTCTTTCC	ATHAMACCICAT	GECACGETC	GCTCACGAAA			TACACACTIVE
	ACCOCCTACG		CANGANTOG	AGACCAAAGG	TACTURECA	CARCTCCGAG	CCACTGCTTT	TCCGACAGGC	ACAGGGGCAT	ATGTCTGAA!:
		Xhol								-
5301	AGAGGCCTGT	CCTCGAGCGG	TOTTCCCCCG	TECTECTEGY	ATAGAMACTC	GOACCACTCT		CICCCGTCCA	OCCCAGCACO	AACCANGCTA
	TCTCCGGACA	OGAGCTCGCC	ACANGGEREC	ACCAGGAGCA	TATICITITICAG	CCTRGTGAGA	CICIOTITICC	CAGCCCCAGGT	ccconcence	TICCTCCGAT
5401	ACTOCCAGOO	GTAGCGGTCG	TTGTCCACTA	GOOGSTECCAC	TURCHCCAGG	MUTCHCANGAC	ACATOTOCC	CTCTTCGGCA	TCMGGMGG	TOATTOSTIT
	TCACCCTCCC	CATCGCCAGC	AACAGGTGAT	CCCCCAGGTG	NACHAGETCC	CACACTTCTG	TOTACAGOOG	GAGAAGCCGT	AGITICCTTICC	ACTAACCAMA
5501	GTAGGTGTAG	OCCACOTGAC	COGGRETICC	TGAAGGCGGG	CTATAAAACG	CONTRACTOR	acenteenee	TCACTCTCTF		GICTGCGAGG
	CATCCACATC	COSTSCACTO	GCCCACAAGG	ACTITICCCCCC	GATATTTEC	COCACCOTOG	CCCAMICAGO	ACTGAGAGAA	GGCOTAGCGA	CAGACGCTCC
5601	OCCAGCTOTT	GOOGTGAGTA	CICCCICIGA	AAAGCGGCA	TEMETICING	GCTAAGATTG	TCAGTTTCCA	AAAACGAGGA	CONTITIONTA	HCACCAGO.
	COGICGACAA	CCCCACTCAT	CARRENGALT	THYCGCCCGT	ACTGAAGACG	CGATTCTMC	ACTCAAAGGT	TTTTGCICCT	CCTAAACTAT	AAGTGGACC*1
							Hingfill	•		
5701	CONTRACTOR	OCCT TIGAGG	PROCECCAT	CCATCTOSTC	AGNANAGACA	ATCTITITION	TOTOMOCTE	TOTICANDETT GOTGOCAAAC		GENCETTIGA
	OCCCCACTA		CACCOGCGTA	GCTAGACCAG	retriencies	TAGANAACA	ACAGTTCGNA	CCACCGTTTIG	CTOCCCATCT	CCCCCAACCT
					Pvul					
5801	CACCACTTO	OCCATOGAGE	GCACKGTTTG	GEFFFFC	CGATCGGCGC	GCTCCTTGGC	CGCGATGITT	ACCTOCACOT	ATTROCOCOC	AACCCACCK.
	GICOTIGAAC		CCTCCCAAAC	CANAANCAGO	CCTAGCCGCG	CCACCAACCC	GCCCTACAAA	TCGACOTGCA	TAAGCGCGCG	THECGTEGO
5901	CATTCOOGAA	AGACOOTGGT	OCCUTCGTCG	GGCACCARST	CCACCCCCCA	ACCIDENTION	TOCAGGGTGA	CAMOOTCAAC	octooreocr	Accretector
	GTAAGCCCTT	TETGCCACCA	CCCGAGCAGC	CCGTGGTCCA	COTTOCOCACT	TOTOCOCONC	ACCITOCOTACT	GITICCAGITIG	CCACCACCGA	TOCAGAGG
6001	GTAGGCGCTC	GITGOTCCAG	CAGAGGCGGC	COCCCTTACC	CGAGCAGAAT	COCCUPACO	GGTCTAGCTG	CONCINCATOR	GOGOGGICHG	CONCCACGO
	CATCCCCCAAG	CAACCAGGTC	OTCTCCCCCG	GCCCGANCTIC	CCTCCTCTTA	CCCCCATCCC	CCAGATCGAC	GCAGAGCAGG	CCCCCAGAC	OCAGGTTO CCA
6101	AAAGACCCCG	GOCAGCAGG	OCCCCTCCAA	GTAGTCTATC	THECATECITY	GCAACTCTAG	CCCCTCCTGC	CATGCGCGGG	COCCAAGCGC	GCCC-TCG-TAT
	THETOGOGE	CCGTCGTCCG	CGCGCAGCTT	CATCAGATAG	AACGTAGGAA	CCTTCAGATC	GCCACACGACG	GTACGCGCCC	OCCGPTCCCG	CCCCACCATA
6201	GOOTTGAGTG	GOGGACCCCA	TOCCATCICAG	TOGGTHANGED	CCGCACGTA	CATCCCCCAA	ATCTCCTAAA	CCTAGAGGGG	CTCTCTGAGT	ATTCCAAGAT
	CCCAACTCAC	CCCCTGGGGT	ACCGTACCCC	ACCENCTOGE	GCCTCCCCCAT	GTACGGCGTT	TACAGCATIT	GCATCTCCCC	GAGAGACTCA	TAAGGITCIA
6301	ATOTAGGGTA	GCATCTTCCA	CCGCGGATATCC	TOCCOCOCAC	GTANTCGTAT		MYSCACCEAG	GACCTCCCCA	CCGAGGTTGC	TACGGGCGG
	TACATCCCAT	COTAGAAGGT	OGCGCCTACG	ACCOCOCOTG	CATTAGGATA	TCAAGCACGC	recenedation		CCCTCCAACG	ATGCCCGCC
6401	CTGCTCTGCT	COGRAGACTA	TOTOCOTOM	GATCACATGT	CALTERGATE				TRACGICTOT	CAGACC-TACC:
	GACGAGACGA	GCCTICIGAT	AGACGGACTT	CTACCCTACA	CTCAACCTAC	TATACCAACC	TOCGACCTTC	TOCAACTTCG	ACCOCAGACA	CTCTGGATG:

Figure 150

PMRKAdSgag MER682

6501	CCOTCACOCA	CGAAGGAGGC	GTACKIAGTCG	CCCACCTTCT	ACTUAL DESCRIPTION OF THE ACTUAL STREET	מבנישמונישעים מבנישמונישעים	ACGRECAGAR A	CCCGCGTCAT	CAGGICCCAA	TCCTTGATCA AGGAACTACT
6601	TGTCATACTT		TTTTTTTCC ANAMAAAGG		OTTGAGGACA	AACTICTICUSC TITSACIANGOS	CCAGAMAGGT	CATGAGAACC	ATCCCAAACC TAGCCTTTGG	CCINCOCCETY :
6701	CGAACGGTAA	GAGCCTAGCA	TGTAGAACTG ACATCTTGAC	GTTGACGGGC	THE TARGETTE ACCEPTED	AKCATCCCTT TCX:TACCCTAA	THETACKGGT	AGCGCCTATG TCGCGCATAC	CCTOCCCGGC	CTTCCGGAR
6801	GAGGTGTGGG	TGAGCGCAAA	GOTOTCCTG CCACAGGGAC	ACCARCACTT TYGTACTGAA	TGACKETALTG ACTCCATUAG	GTATTTGAMG	TCAGTGTCGT	CCCATCCCCC	CTGCTCCCAG	AGCAAAAAGT TCGTTTTTCA
6901	CCOTCCCCTT	AAACCTTGCG	CCTAMACCGT	CCCCCTTCCA	CTGTAGGAAC	AMGAGTATOT	TTY CCCCCCCC	ACCCTATTC	TTGCGTKTTGA	TGCFGANGGF ACGCCTTCCT
7001	TCCCGGCACC AGGGCCGTGG	TCGGAACGGT ACCTTGCCA	TOTTANTTAC ACANTTANTG	CTCCCCCCCC	ACCARCATET TEGITGETAGA	CCTC AAAACC GCACITTICGG	CANCTACAAC	TCCCCCACAA ACCCCCTCTT	TGTAAAGITC ACATTTCAAG	CAAGAAGCGT GTTCTTYCGCT
7101	GGGATGCCCT	TOATOGAAGG	CAATITITIA	AGTICCTCGT TCAAGGAGCA	AGGTGAGCTC TCCACTCGAG	TTCACOCCAG ANGTECCECTC	CTCACCCCT	CCTCTCAAAG CCAGACTTTC	GGCCCAGTCT	GCAAGATHAG CGFTCTACTK:
7201	CCAACCTTCG	CHCCANTGAG	CTCCACAGGT	CACTOOCCAT	TAGCATTTGC ATCGTAAACG	ACCACCAGCG	GAMAGGECT	AVACTOGCGA TTTGACCGCT	CCTATOCCCA	TTTTTTCTGG AAAAAGACC
7301	CCACTACCAG	TACAAOGTAA	OCCORTOTTO COCCCAGANC	MAGGICCC	TCCCATCCAA AGGGTAGGTT	CCANACGCCG	TAGGITCITCGC	GCGGCAGTCA CGCCGTCAGT	CTAGAGGCTC GATCTCCGAG	ATCTCCGCCG TAGAGGCGGC
7401	AACTTCATGA	CCACCATGAA	CCCACCANC	TOCTTOCOA	AGGCCCCCAT	CCAAGTATAG	GTCTCTACAT	CGTAGGTGAC	AAAGAGACGC	TCGGTGCGAG
	TTGAAGTACT	COTCOTACT! Pwd	cccenecres	ACGNACCCTT	TCCGGGGGTIA	GGFTCAFATC	CACAGATATA	CATCCALTG	וורורופני	אחררארתר
7501	GATGCGAGCC	GATCGGGAAG	AACTGGATCT	CCCGCCACCA	ATTECHACAG	TOCCTATTCA	TCTCCTCAAA	CARCITICAGE	CHOCGACOCO	CCGAACACTC
7601	CHACTORET				TOCACGARCT	GTACATECTO	CACCACCTTG	ACCTGACGAC	CGCGCACAAG	GAAGCAGAGT
•	CACGACCGA	AACATTITIG			ACCENCOCCIA	CATGTAGGAC	CTCCTCCAAC	TOGACTOCTG	TOGACTOCTG GCGCGTGTTC	CTTCGTCTCA
							Li Li Marini di Li	ALL STATES	CACCACACAT	ACCESTIVES ATV
10//	CCCTTAAACT	r coccadece	ACCCCCCAAA	CCGACCACCA	GAACATCAAG		CETAACTURICA	GACCGACGAG		TOCCACCTA
7801	OGACCACCAC	GCCGCGCGAG	CCCAAAGTCC	AGATGTCCGC	GUACTACCANT	CTSTACCTIVEA	TCACAACATC	CCCCAGATOG	CAGCTOTCCA	TOGTCTGGAG ACCAGACCTC
	C-I William	י נפתותהיור		•						
1901	CHCCCGCGGC			_		-	(3OCOCO:UOC)			Trechedene.
	GAGGGCGCC	3 CAGTCCAGTC	CGCCCTCGAG	GACGTCCAAA	TOCACCCTAT		CHACCCAGTC CCCACCCCA Kom	TCTAGGTCCA	CTATIGGATTA	AAGGICCCCG
RNO1	E HE E HE EAT	rasometer.	CASTINATANG	AGGCCGCATC	נבנישנישיניני		פארידאריני הייארארינינט	COCCACTOGGC	COCHOTOGIC COCOGGISTIG TECTTERATE	TCCTT(RCATF:
))	ACCAACCACC		CCCCCAGCTA CCCAACCTTC				בופאומכנאד מסכמכמכנסכ	CCCCCACCCG	OCCUCACAC	ACCIANTETAL

8101	ATGCATCTAA	MCCGCTCAC	GCCCCCCAAC	נוננונאנאאנון	AGENTARICE	כנישיעכבנישב	COCCAGACAC	GCCAGGGCA	CONCORROCC	geneceopur
] ; !	TACGTAGATT		בפכיכנוניונים	GREGORICA	TOTAL	CHICCHAGGGG	ואטנבונבונכב			כפכנונומכבניו:
8201	ASSASCTACT TCCTCGACCA	OCTODOCOCO COACGCGCGC	PARCETTOCTC ATCCAACCAC	GCCTTCCCT	נייאת האימיה מייונאית ניימי	CAACTAGAGG	TOANTOTYNG M. T. PAGAGEG	CCCTCTCCCT CCGARCGCA	CAAGACGACG	CCCCCCATTCA
8301	GCTTGAACCT		TEGACAGAAT	CAAITTCGGT	CHATTAGACG	מאשנינינוגאנינ	GENNANTETE			CTTCMTAGG:
	CGAACTTOGA	CTTTCTCTCA	AGCTGTCTTA	GTTAANGUEA CAFETAACTEE	באיביאאכיזיגיב	CCCCCCCACCC	CCTTTTAGAG	GACGTGCAGA	GGACTCAACA	GANCTATICC!
				£	- Table					
8401	GATCTCGGCC	ATGAACTOCT	CONTCICTIO	CHOCHNOAGA TCHCCGGTG	TCTCCGCGTC	CONTINUE	CACOGINGCG	GCGAGGTCGT	TCGMATGCG	GCCCATCA"
-	CTAGAGCCGG	TACTTGACGA	GCTAGAGAAG	האתאארכידכיד	אמאראונימראק	GCCCAGCGAG	CITCCCACCCC	COCTCCAGCA	ACCTITTACGC	CCC37TACTC":
8501	TOCCAGAGG	CONTIGACKEC	recenceme	CAGACGCCCC	TKTTAGACCAC	פניבכבבידוכם	GCATCGCGTG	COCGCATIGAC	CACCTGCGCO	AGATTCACC "
	ACOCYCTICC	GCAACTCCGG	AGGGAGCAAG	פתכתמכמככם	ACAINCTIGGTG	CLYCOCKONNC	CCTAGCCCCC	GCCCUTACTG	OTGGACGCGC	TCTAACTCG.
8601	CCACGTGCCG	GOCGANGACO	GOCGNAGACO OCOTAGITITIC	GCAGGCGCTG	AAAGAGGTAG	TYCACACACTURE	recenence	TTCTGCCACG	AAGAAGTACA	TANCCCAGC:
	GOTOCACOCC	CCGCTTCTGC	CCCCTTCTGC CCCATCAAAG	CONCCOCGAC	THICHCOATC	AACTCCCACC	ACCECCACAC	AAGACOGTOC	TECTICATOR	ATTICGGTCCK:
		ŭ į	EcoRV							
8701	TCGCMCGTG		GATTCOTTGA TATCCCCCAA	OCCUTCAAGG	CGCTCCATGG	CCTCGTAGAA	GTCCACOGCG	AACTTICAAAA	ACTOSOAGIFF	CCCCCCCC
	AGCGTTGCAC	CTRACCAACT	ATAGGGGGTT	CCCCAGTICC	GCGAGGTACC	GGAGCATCTT	CAGGTGCCGC	TICAACTETT	TGACCCTCAA	COCCCCCCTC
1088	ACCEPTANCE	CCTCCTCCAG	MGACGGATG	AGCTCGGCGA	CAGTATCOCG	CACCTONORS	TCANAGGCTA	CAGGGGGCCTC	TICTICTICT	TCAATCTCC:
! ! !	TOCCARTICA	CCACCACCTC		TCGAGCCGCT	GTCACAGCGC	GTKIGAGCGCG	AGTITICCGAT	GTCCCCGGAG	AACAAGAAGA	ACTTAGAGGA
									British	1
8901	CTTCCATAAG	GECTECECT	refrencer	CTOCCOCCGC	TRICACCACAC	GCGACACAGC	GCCCACGACG	GCGCACCGGG	AGGCGGTCGA	CAMAGEGETE
1	GAAGGTATTC		AGANGANGAN	CANCECCCCC	ACCCCCTCTCC	CCCTGTGCCG	CCGCTGCTGC	COCCTOOCCC	TCCGCCAGCT	GFTFCGCGAG
9001	GATCATCTCC	CCGCGGCGAC	CCCCATCGT	CICGOTOROG	GUGGGGGGGG	1111000000	GCACAGTTOG	AAGACGCCGC	CCGTCATGTC	CCCASTTATOS
•	CTACTACAGO	_	CCGCGTACCA		CGCGCCCAACA	AGAGCGCCC	CCCCTCAACC	TTCTGCGGCG	OCCAGTACAG	GCCCAATACC
9101	GFTGGCGGG	COCTOCCATG	CGCCAGGGAT	ACCCCCTAA	CGATKICATCT	CAACAATTGE	Transtracta	CTCCGCCGCC	GACCCACCTO	AGCGAGTCT
	CAACCGCCCC	CCCACGGTAC	GCCGTCCCTA	TOCCGCGAIT	GCTACGTAGA	GFTGTTAACA	ACACATCCAT	GAGGCGGCGG	CICCCIOGAC	TCGCTCAGG
			Xhol							
9201	CATCUACCOG	ATCGGAAAC	CTCTCGACIAA	AGGCGTCTAA	CCAGTCACAG			CETGGCGGGC	GOCAGCGGGC	GCCGGTCG(X:
	GTAGCTGGCC	TAGCCTTTTO	GAGAGCTCTT	TCCGCAGATT	GGTCAGTGTC	ACCCTTCCAT	CCCACTCGTG	GCACCGCCCG	ccencecced	CCGCCAGCCC
							Manage Manage	•		
9301	GINGITICIO	GCGCAGGTGC	TOCTGATGAT	GTANTTANAG	TACKSCAPICT	TICAGACTICC	GATOCTICAC	AGAAGCACCA	infrectificed	TCCGGCCTCC
	CAACAAAGAC	COCCTCCACG	ACGACTACTA	CATTANTTEC	ATCUTACORGA	ACTICTOCCGC	CTACCAGCTG	remediagr	ACAGGNACCC	AGGCCGGGACG
9401	TGAATGCGCA	OCCOUNTCOCK	CATYCCCCAG	GUTTCCTTTT	CACATORICO	CAGGINCTITES	-	GCATGAGCCT	TICTACCOCC	ACTICITICS
	ACTTACGCGF	CCCCAGCG	GTACCOCCTC	CGANGCANAA	CITATIACICCOC	GTCCAGAMAC	ATCATCAGAA	CCTACTCGGA	AACATOGCCO	TGANGNAGA
9501	CICCINCCIC	: TTGTCCTGCA	TCTCTTGCAT	CTATCECTOC	נאגנטטטטטטט	CAGTITAGAT	CTACKTTGGCG	CCCTCTTCCT	CCCATGCGTG	TGACCCCGAN
	GACGANGGNG	AACAGGACGT	AGINGANCGTA	GATACKGACG	בנגאכנוכניפכ	CICANACION	כאדככארכככ	COCACARACIA	CEGTACCECAC	AC17GGGGCT I
9601	GCCCCTCATC	: GOCTGAAGCA	CASICTAGGTC	GGCGACAACG	COCTROSCEA			GTCAGGGTAG	ACTORANGEO	ATCCAPATICY.
-	CCCCCACTAG	CCGACTTCGF	CCCGATCCAG	CCCCTGTTGC	CCCACCCCAT	TATACCGGAC	GACGTGGACG	CACTCCCATC	TCACCTTCAG	TAGGTACAGG

Figure 15F

				TACE GEOTGETAR				SACGE CENTENCES				TORGECEGAC GEGEGAACCG ACTEGGGCTG CGEGETTGGC Handin	AAAGTTTTT CGAAATTGT	
CTGCCIAGAGC GACGCTCTCG	GGCGGCGCT	TCCAGGTGAT			CHEMICCCC				-				-	
TTAACHETET (KOTTACKES) ANTTECCASA CCACTERISCE	CANANAOTICC GITTITICACG	-								-	r coccadococo a cacacitoacoc	3 AGGAGGACTE C TCCTCCTGAA		C GCCCTGGGGG ANACCCCANAGGGGTTT G CCCCGCCCCCG TTTTGGGTTTT A ACATAGTAGA GCCCGGGGGCCCGGTTTTGGGTTTTTGGGTTTTTGGGTTCCCCGGTTCCCCCGGTTCCCCGGTTCCCCGGTTCCCCGGTTCCCCGGTTCCCCGGTTCCCCGGTTCCCCGGTTCCCCGGTTCCCCGGTTCCCCGGTTCCCCGGTTCCCCGGTTCCCCGGTTCCCCGGTTCCCCGGTTCCCCGGTTCCCCGGTTCCCGGTTCCCCGCTCCCCGCTCCCCGCTCCCGGTTCCCCGCTCCCCGCTCCCCGCTCCCCGCTCCCCGCTCCCCGCTCCCCGCTCCCCGCTCCCCGCTCCCCGCTCCCCGCTCCCCCGCTCCCCGCTCCCCGCTCCCCGCTCCCCGCTCCCCGCTCCCCGCTCCCCCC
	ACCASTRAT GETATCCCAC TXSTICATIA CCATAGGGTG FOSTIV			-			-	-	F CCTCCTACCG	S AGGAGGGCGA C TYCTYCYCGCT	A GAACCTGTTT T CTTCCCACAAA	O TTGCTGCGCGCCCCCCCCCCCCCCCCCCCCCCCCCCCC	-	A CTTTGTANGC T GANACATTGG G GCCTGCTNA
AACGGAGTAG TTGCTOSTC		מאנהאז ככסכידי			-	TOTACCOCOCOCOCOCOCOCOCOCOCOCOCOCOCOCOCOC	CCCCCGGTTC		A A C C T C C C C T T T T C C C C T T T C	CHICACTTOS GACCTGAACC	S ACCIDENTAL	A TEGEGRACES F AGEGETEGE		C ATCHETERAN G TAGACACCET C ATTCACAGAT G TAAGTCCCTA
AGTTROGECAT TCAACCCRITA	GETTERGEGG		TGTTRECRENC ACAACGCGTC		CGCTTACCOC	AMACCOSTG	ACTICIOCACIÓN TEMACIGICAT		CATCACCCC	CCGCCACTAC GCCCGTGATG	r caccectage	2 ATCCCCTCTAA		A GCACTGACTAC T CCTGACTACG G ACAACGACGC C TGTTGCTCCG
GATATHUAGU	CCTTACTCCTTT GCATCACYCAA Nytti	CCCCTCTAGA	CCKTTCC AGA GCC AAGGTCT	AGAGCCTCTA TCTCGGACAT	GTCATCCATG	GCGCTAGCTT CGCGATCGAA	CCAAGOGTTG	TTCCTCCGGA AAGGAGGCCT	CAGCGGCAGA	בכשכנונפכבבנו	TOATACGCGT: ACTATGCGCA	GAGCTICACIONS:	COCCOCCACCT COCCOCTOGA	A GGTGGCTAFA T CCACCGATAT G CACAGFAFFF C GTGTCGTCCC
GTGTTGATG	GTARGECETE GAGTEAATA CATTEGGGAG CTCAGTTTAT	GCCCTCCCP.SG CCCCGAGGCCC	GTCHCGGACG	OTOCANAAGO CACOTITICO	GCCGFCCFCC CGCCAGGCGG	OCCCCTCCTACTACTACTACTACTACTACTACTACTACTAC	CCCANTARAR	CCCTTCCAAA	AGAGCAAGAG TCTCGTTCTC	GAACCCCCGC	AGCTGAAGCG TCGACTTCGC		A CACGTEGOGG	CCCCCCCCC CCCCCCCC TATAGTCCCC A ATATCACCCC
GCTATGCGCC CI CCATACGCGG G	GTAAGCCCTC CATTCGGGAG	AGCCADCCC TCCCADCCGC	CCCCCCCCTTF CCCCCCTTF Xbal	CCACATCTOG	CCCGTATCCG GGCCATAGGC	TTCCAGGGGC AAGGTCCGCG			AGCAGCGGCA TCGTCGCCGT		CCANGGGTGC		cococococh secocococot	A TECENACAC E AGENTIFICET D TECACAGGA
ACAAAGCOST	TGAGACGCCA ACTCTGCGCT	CCCCOTCCCA	GTCGTGGAGG	AATCGTTGAC TTAGCAACTG	CCCAAGCTCG	MACCOMO	COAGCGAGGG	CTCCCCCTCA	CCCCCTCCTC	COCCAGCAGA	TCACCCCCAC	ATCCCCCTAG	CCTAATCAGG	CCACOTOCGT OOTGCACOCA CTCATOCGGC GAOTACCGCG
9701	9801	1066	10001	10101	10201	10301	10401	10501	10601	10701	10801	10901	11001	11101

11301	TCCATTTCAT	_		TOURSE	נונימ-אשבעני		ACAMOSTRAGE	CCCCATCAAC		TTAGCCTCGG
	ACCTARACTA	TITICTACCAC	CTCTCTTATC	ACITACI TINCT	C(#1:11 T.AAC	TATEORETICAE	TETTECACEG	GCCKTAGTTG		ANTICACITY
11401	CAACTITIAC	GCCCGCAAGA	TATACCATAC	CCCTTACGIT	CLT.ATA:ACA	ACCIPACITANA	ひとしいいしている	TTCTACATGC	CCATCCCCCT	CAACTTTCT .
	GTTCAAAATG	COGCGTICT	ATATECTATE	GOGAATGCAA	CATTENITY INTE	TITANITA	כדאייכדכיניב	AAGATGTACG	COTACCGCGA	CHTCCACGV 1
11501	ACCITICACCG	ACCACCTOCG	CGTTTATCCC	AACGACACAC	TEXTANGE	CHILDWICHE	M TO COGGING	GCGAGCTCAG	COACCOCCIAG	CTSATTSCACA
	TOCAACTCGC	TOCTOGACCC	GCAANTANCO	TRICICGCGT	ACKSTICTTACG	GCACTOGGANC	TYPOCCCARTO	COCTCGAGIC	GCTOGCCGCTC	GACTACOTUT
11601	GCCTGCAAAG	acceptaget	GGCATTRIAGA	GCGGCTATAG	NUNDCUCUNG	TCCTACTTIG	ACCCCCCCCC	TGACCTGCGC	TOGGCCCCCAA	GCCTAACTICGC
	COGACOTITIC	CCGGGACCGA	CCGFFCCCGT	COCCCACTATE	TUTECOCOL	ACCATGAMAC	TOCOCCCOCG	ACTEGACOCC	ACCCGGGGGTT	COOCHOCOC:
11701	CCTGGAGGCA	acrossoccs	GACCTYRGCT	CACCATA COCA	בבבניניניניניניני	CTGCCAACGT	COCCACCORG	GACCAATATC	ACCAGGACGA	TONCTACGAG
	COACCTOCGT	CCACCCCCCC	CTOGACCCCA	CCCCCALCUL	202020202020	GACCGTTGCA	DUCCECCCAC	CTCCTTATAC	recreerer	ACTICATICCTIC
									Pstl	
11801	CCAGAGGACG	GCCAGTACTA	ACCUPATO ATC	THETERATEA	CATCATGCAA	GALFICANCIDG	ACCORREGART	occooccoc	CTGCAGADCC	AGCCGTCCC :
	GENERACTICS	CECTCATGAT	TCGCCACTAC	AAAGACTAGT	CTACTACGIT	CTGGGTTTTCC	TGGGCCTX.CA	CCCCCCCCCCC	GACGICTCOO	TCGGCAGGC
11901	CCHTAACTCC	ACGGACGACT	GGCGCCAGGT	CATGGACCGC	ATCATCTCCC	TYPACTYCCATO	CAATCCTYAC	GCGTTCCGGC	AGCAGCCGCA	GCCCAACCO'!
	GGAATTGAGG	TOCCTOCTGA	CCCCCCTCCA	GTACCTGGCG	TAGTACAGCG	ACTGACGCGC	GTTAGGAC.TG	CCCANCOCCC	restedeest	CCOCITIOCC
							Pyrid			
12001	CTCTCCGCAA	TICTOGARGE	OCTORPOCCO	GUGUGUGCAA	ACCCCACGCA	CGAGANGGTG	CTGGCGATCG	TAMACCICCT	GCCCGAAAC	AGGCCATC
	GAGAGGCGFT		-	COCGCGCGTT	TOGGOTOCOT	GCTCTTCCAC	GACCGCTAGC	ATTTECCCCA	CCGCCTTTG	TCCCGGTAG
12101	GGCCCGACGA	GOCCOGCCTO	GTCTACGACO	CYCTGCTTCA	CCCCCTCCCT	CCTTACAACA	GCGGCAACGT	GCAGACCAAC	CTGGACCGGC	TOTOGOGGA
	CCOOGCIOCT	CCCCCCCCCC	CACATGCTGC	GCGACGANGT	COCCOCYCCGA	GCANTISTINIT	CCCCCTTCCA	corcrostris	GACCTGGCCG	ACCACCCCC"
12201	TOTOCOCCAA	accordacac	AGCGTGAGCG	CACCCCAGCAG	CAGGGCAACC	TOCOCTUTAL	GGTTGCACTA	AACOCCTICC	TGAGTACACA	OCCCGCCAN'
	ACACGCCCCTC	COCCACCOCO	TCCCACTCGC	GCCCOTCCTC	GTCCCGTTOG	ACCCCAAGGTA	CCAACGTGAT	TTGCGGAAGG	ACTCATGTOT	COCCIOCOTTO
12301	OTOCCOCO00	GACAGGAGGA	CTACACCAAC	THYSTCACCO	CACTGCGGCT	AATGGTGACT	מאקאכאכנככ	AAAGTGAGGT	GTACCAGTCT	GCCCCAGACT
	CACOOCOCCC	CHOICCICCT	CATGGGGTTG	NACACTOGG	CTCACGCCTA	TTACCACTGA	CTCTGTGGGG	TITICACTICCA	CATOGICAGA	CCCGGTCTGA
			Psi	_*						
12401	APPRIMECA	GACCAGTAGA	CAAGGCCTGC	AGACCGTAAA	CCTGAGCCAG	GCTTTCAAAA	ACTITICCACACE	GCTCTCGGGG	GIGCGGGCTC	CCACAGGGGA
	TAMAMOGT	- CROSTICATOR	GTTCCGGACG	TETOGENETE	GGACTCGGTC	CHAMBETTE	TGAACGTCCC	CCACACCCC	CACOCCCGAG	CONCINCESCY
12501	CCCCCCCACC	GIGICIAGET	TOCTICACOCC	CAACTERICITIE	CTGTTCX-TCC	TRICTANTAGE	GUTTETTO	GACAGTGGCA	OCO TOTOCCC	CGACACATAC
	GCCCCCTCG	CACAGATCGA	ACGACTICICES	CITICAGCGCG	מאכאאכמאכמ	ACGATTATCG	CTATANGTER	CTCTCACCGT	CGCACAGGGGC	CCTGTGTATG
12601	CTAGGICACT	TOCHOACACT	- GTACCGCGNS	GCCATAGGGTC	AGGCTGCATGT	CSTALIGABICAT	ACTITICOMOG	AGATTACAAG	TGTCAGCCGC	CCCCTNOOCC
	GATCCAGTGA	ACCIACTICITICA	· CATGGCGCTC	COCTATCCAG	TCC(CCTACA	CCTOCTCGTA	THIMMOGREC	TCTAATGTTC	ACAGTCGGCG	COCCOVCCCC
								ţ	Print	
12701	AGGAGGACAC	: accenacete	S GAGGCAACTC	TAMACTACCT	CHITACTIAAC	CONTRACTOR	AGATCCCCTC	GITTGCACAGE	TTAMACAGCG	ACCARRANCE
	recreated	cccorcoorc	: Crecommen	ATTIMINATORA	CCACTOSTING	CCCXCCTCT	TETACKORING	CAACGTGTCA	ANTIFICATION	Techecution
12801	CATTITIOCOC	: TACCTRICARC	: ACACCCTITAG	CCTTANCCTG	ATTACCACT	GINTLY	CARCITICATE	CTRICACATICA	CCCCCCCCCA	CATCGAACCC
	GTAMACGCG	3 ATGCACGTCG	TCTUTACACTIC	GGAATTGGAC	TACTACTACT	CCCATTGCGG	מובנאניעניניני	GACCTIGITACT	CGCGCGCGTT	GTACCTTGGC

Figure 15H

12901	GECATURATO	CCTCAAACCTI (TTTCACCAAT	CCTATCTTCA
	CCGTACATAC	GGAGTITGGC (CCCCAAATAG .	TTCXXXXXXTT /	MY THINH O				V 1001000	
13001	ACCCACACTO						ANGATGGATT	CCTCTCTCCCAC	CACATAGACG	ACACACTATATATATATATATATATATATATATATATAT
	TOCCOTONC	CGATGGCGGG (GGACCAAAGA .	איזעאיכניניכני	TAAGGTICCAG	TARE TALLAI				
•					CAGCAGGCAG	אימשנימינים י	CCCAANCGNA AGCTTCCCCA		GGCCAAGCAG	CTICTCCGAT
13101	AAGGGGCGTT						CUCHTICCT	TCGAAGGCGT	CCGGFTCGTC	GAACAGGCT'.
					1 limitit					
13201	CTAGGCGCTG	במסכבכבמם	OTCAGATGCT	ACTACCCCAT				CTCGCACCAC	ccccccooc	CTOCTORGO:
	GATCCGCGAC	363366666666666666666666666666666666666	CAGTETACGA	TCATCGGGTA	AAGCTTC(:AA (CTATCCCAGA	GAATGGTCGT	GAGCOTGOTG	GCCGGCCGCG	CALGALLLI
			Psil	*						
13301	ADGAGGAGTA	CCTAAACAAC	TCGCTGC11GE	אשכננפכעפנפ			CATTROCCAA	CAACGGGATA	GAGAGCCTAG	TOGALANIA
	recreetest	OGATTHOTH	AGCGACGACG	TOSKETICE	CCTTTTTT		GTAMGGGTT	GITGCCCTAT	CICICAGAIC	
13401	GAGTAGATGG	AACACGTACG	CCCAGGAGGA	CAGGGACGTG	CCNARCCCCC		COGICCICAN		GECAGCOGNG	Terestere.
			acercencer	GICCCIGCAC	COLUMN TO SANGE	CCASCOCIACIO	GOCAGCAGTT		CAGICOCCCC	
13501	GACGACGATG	ACTOGGGAGA	CGACAGCAGC	GTCCTGGATT	Technerand		TTTCCGCACC		GCTGGGGGGAGA	
	CTCCTGCTAC	TGAGCCGTCT	GCTGTCGTCG	CAGGACCTAA	ACCURACYTO	ACCCTTROCCC	AAACCCCTCG	AAGCGGGGTC	CGACCCCTCT	TACAMAITT
13601		GCATGATGCA	AAATAAAAA	CTCACCAACA	CCAMPACACC	CACACATTAGAT	Tricitistat		ATGCGGCGCG	CCCCCATCTA
		COTACTACGE	TETATETET	GAGTCGTTCC	GGTACCCCTGG	CHICCAACCA	ANNCHACATA	ACCCCANTCA	TACGCCGCGC	GCCGCTACAT
11701		. مصاددات	CCTACCACAG	TOTOGRANGE	CHITANICALAG	TEACHOCKARE	GCTYGGTTCT	CCCTTCGATG	CTCCCCTOGA	CCCCCCTTT
		GCAGGAGGGA	GGATCACTICTIC	ACACCACTCG	CGCCCCGGTC	ACCOCCOCCG	CCACCCAAGA	COGARGETAE	GAGGGGGACCT	GGCGGCM.
		Koni								
1 and	Chart Miles	GGTACCTGC	GCCTACCOCC	CHECHGNANCA	CCATCCCTTA	CICTGAGITG	CCACCCCTAT		CCGTGTGTAC	
		CCATGGACGC			COTAGGCANT	GACIACTICIAAC			GCCACACATO	
13901	ACAMGITCANC	GGATGTGGCA	TOCCTIGNACT	ACCAGAMAGGA	CCACACCAAC	THICHGACCA		-		
		CCTACACCGT		TEXTECTION	GENETICETTO	AAAGACTGGT	CCCAGTAAGT	•		
14001	_	. ATCANICITO	ACGACCGGTC	GCACTGGGGG		_			TCAACGAGTT	
	GIGHOTOTO	TACTTACAAC	TECTOCCCAG	CIGTGACCCCG	CCGCCTCGACT	TITCGTAGG				-
14101		AGGCGCGGGT	GATOCTOTOG	COCITICCCTA	CTAAGGACAA	-			_	
t t		TECGOGGCCA	CTACCACAGC	OCCUACOGAT	GATTCCTVTT	ACTECACTTC	GACTITATIGE	TCACCCACCT	CAAGTGCGAC	פפכורררפו
				•	Paul			•		
14201	ACTACTOCOL	GACCATGACC	ATAGACCITA	TRANCANCAC	GATCHTYKAG				-	
i I		r CTOGTACTOG	TATCTGGAAT	ACTINGTING .	CTACK:ACK:TC	CTCATGAACT			_	
14301	-	r GACACCCGCA	ACTICAGACT	GOTTITIES.	CCCMCACTG					
1	CCATTTCAA	A CTGTGGGCGT	TCAAGTCTGA	י כבכריאאונידוי	האטראקדויאה					
14401	ATTITICATE	C CACCATGCGG							CCAGGAGGGC	TITING ALCH
	TAAAACGACG	G GTCCTACGCC	CCACCTGAAG	recentences	COCACTECITY	מאכאכככם	TAGREGITES	CCGFRAGGAA		

Figure 15I

PMRKAd5gag MER682

14501	CCTACGATGA	TCTGGAGGGT	CCTAACATTC	CCCCACTICAT	CHATCHER	GITTALICAGE	CCACCTTGA	AGATGACACC	GANCAGGGCG	COCCIONEGE
•	CGATGCTACT	AGACCTCCCA	CCATTGTAAG	GOCCHUNCAA	CCTACACCTG	CCSSARGERY C	CCTCCAACTT	TCTACTOG	CTINGTOCOGC	כככבעכיכנים
14601	AGGICGCCAGC	NCAGCAGTG	CCAMPAGGGG	ההתאתאתאת				GTOGAGGACA	TCAACGATCA	TOCCATTON:
	1ccccco1cg	TIGICGICAC	CONTRACTOR	CCTICICTIC	NOCETIVE CONTR	נאגאאיעאינפ	דדאכיהדי:יאמכ דיי	CACCTCCTGT	ACTIGCTAGE	ACCCTANGLO
14701	GCCCACACCT	TYGCCACACO	CCCTCACGAG	AACCCCCTC	AGGGGGGAAAG	NXTOTATION	פו: בניו בכניו בכ	CCCCTCCCCA	ACCCGAGGTC	GACAAGCCTF
	CCCCTGTOGA	AACGGTGTGC	CCGACTCCTC	TYCGCCCGAC	TCCCRCTTCG	TCCCCTAKTT	CCACCCCAGG	GACGACGCGT	TGGGCTCCAG	CICTICGGW
										Ę.
14801	AGANGAAACC	GGTGATCAAA	CCCCTTACAG	NYTHUNDOWN	CAAACCCAGT	TACAACCTAA	TAMOCANTON	CAGCACCTTC	ACCCAGINCC	GCAG("TG/"")
	1CT ICT TIGG	CCACTAGITT	GGGGACTGFC	Techorent	CTITICOGREA	ATCITICGALT	ATTCGTTACT	GTCGTGGAAG	TOCOTCATOS	CCTCGACCAT
	Š									-
14901	CCTTGCATAC	MCTACCCC	ACCCTCACAC	COCHATICOC	TEATGGATEC	TOCTITIOCAC	TECTIONCOTA	Accretedent	COCAGCAGGF	CTACTROTO:
¦ !	GGAACGTATG	TTGATGCCGC	TOGGAGICTO	GCCTTACACC	AGTACCTINGG	ACTIMAACGTG	AGGACTICAT	TOGACOCCGA	accreateca	GATGACCACIC
15001	TTGCCAGACA	TCATCCAAGA	CCCCGTGACC	TTCCCCTCCA	CGCGCCACAT	CARCAACTIT	CCGGTNCTVG	GCCCCCAGCT	ornoccepto	CACTCCAAGA
	ACCOUNTS		GGGCACTIGG	ANGGCGAGGT	GUGCCCCTCTA	GTCGTTCANA	GRECACCACC	CGCGGCTCGA	CAACGGGCAC	GIGAGGITTCT
										Asci
15101	OCTICIACAA	COACCAGGCC	GICTACTCCC	AACTCATCCG	CCAGITITACC	TCTCTGACCC	ACCTICITICAL	TCGCTTTCCC	GAGAACCAGA	TITITICOCCIA 73
	CGAAGATGIT	GCTOOTCCOO	CAGATCAGGG	TTGAGTAGGC	COTCANTGG	AGAGACTOGG	TOCACAAGIT	AGCGAAAGGG	CICTIGGICT	AAAACCUCC:
	P									
15201	CCCOCCAGC	CCCACCATCA	CCACCGTCAG	TCAAAACGTT	CCTACTOR	CAGATCACGG	GACCETACCE	CTGCGCAACA	GCATCGGAGG	AGTCCAGCOA
	GOCCOOTCOC	COCTOCTACT	OCTOOCIANT	ACTITITICICAA	GGACGAGAGT	GTCTAGTGCC	CTOCCATOCC	CACCCCTTGT	CGTAGCCTCC	TCAGGTCGCT
15301	GTGACCATTA	CTGACGCCAG	ACCCCTACACC	TGCCCCTACG	TTTACAACGC	CCTGGGCAFA	GICTICGCCGC	CCCTCCTATC	CAGCCCCCACT	TTTTGAGCAA
	CACTGGTAAT	DACTGCGGTC	Tecesectes	ACCCCCCATCC	AAATIGTTCCG	CHACCCCTAT	CAGAGCGGCG	COCAGGIATAG	CTCGGCCTGA	AAAACTKGGFF
15401	GCATGTCCAT	CCTTATATCG	CCCAGCAATA	ACACAGGCTG	האארכי ו מכמכ	TTCCCAAGCA	AGATGTTTGG	COGGGCCAAG	AAGCGCTCCG	ACCAACACCO .
	COTACAGGTA	GGAATATAGC	GGGTCOTTAT	TGTGTCCGAC	CCCOGACGC	AACGCTTCIT	TCTACANACC	GCCCCGGTIC	TTCGCGAGGC	TGGT INSTIGAT
15501	Agreement	COCOGOCACT	ACCOCOCOCC	CTOCCCCCC	CACAAACGCG	GCCGCACTAG	מבטבעכבעכב	GTCGATGACO	CCATCGACGC	COLUMN TO THE CO
	TCACCCCCCAC	GCCCCCGTGA	1000000000	GACCICGCGC	GROTTINGCGC	COCCCTCACC	ccccrccrcc	CAGCTACTIC	GGTAGCTGCG	CCACCACCT
15601	GAGGCGCGCA	ACTACACGC	CACOCCCCCC	CCAGTOTICA	CACTCGACGC	COCCAFTICAG	ACCORDING	GCTGAGCCCC	GCGCTATOCT	AAAATGAACA
	CTCCGCGCGG	TCATGTGCGG	GTGCGCCGGT	GUTCACAGGT	GICACCTIGCG	CCGCTANGIC	TCCCACCACG	COCCTCOOOC	CCCCATACGA	TITINCTIC
15701	GACCOCCCCACAC	GCGCGTAGCA	CONCICCACC	CCCCCCAACC	CCCCACTCCC	GCCMCGC	فصوصوصون	CCTGCTTAAC	CGCGCACGTC	GCACCTATICT
	CTGCCGCCTC	-		COCCOCCTOG	CCCCTGACGG	COCCITOCOC	COCCCCCCCCC	GCACCAATTG	OCCOUNTICAG	COTOCCOOC
		SHI								
15801	ACOGGCCCCC	ATGCGGGCG	CTCGAAGGCT	GOCCOCONCT	ATTRITCACTO	TRECORDECAG	CTCCACCCCA	CGAGCGGCC	CCCCCAGCAGC	COCCACATT
	100000000	TACOCCCCGC	GAGCTTCCGA	CCCCCCCCCA	TAACAGTYIAC	ACTAROGRAPIC	CAGGTCCGCT	OCTORICO GOO	GGCGTCCTCG	GCGCCGGTAA
15901	AGTOCTATGA	CTCAGGGTCG	CAGGGGGAAC	GRUTATIVAGO	TOCOCGACTC	CONTRACTOR	CTREGERATOR	CCGTGCGCAC	ددعددددده	CISCAACTARIA
	TCACGATACT	GAGTECEAGE	GICCCGGTG	CACATAACCC	ACGCGCTCAAA	CCANTCRCCG	GACCCTX:ACG	COCACOCOTO	0000000000	GCGTTY:ATCT
16001	TTGCAGGAA	NAMCTACTTA	GACTCGTACT	CITICITATUTA	TECAGGGACG	CHARTERITA	ACGANGCTAT	GTCCAAGCGC	AAAATCAAAG	ANGAGATRET
i I	ACCTICITY			CAACATACAT	AGGTCGCCGC	CYCCGCGCGT	TGCTTCGATA	CAGGITTCGCG	TITITAGITITE	TTCTCTACGA

Figure 15J

		Parameter .	******							E 400th Total
16101	CCAGGTCATC	GCGCCGGAGA	GCGCCGGAGA TCTANTOOTCE	CCCGANGAAG		ATTACAACT" (CANADA LI SALE
	COTCCACTAG	COCCOCCTICT	AGATACCCGGG	COCCTICATIO	בידור ידי להורכ	באאדידדנינין ני	CCTTTCGAT	TITUTOTOTOTOTOTOTOTOTOTOTOTOTOTOTOTOTOTO	TITITI	
							•	ş		
							CACTOS ANG	COOL	AAAACCTICIT	"TTCCCACC"
16201	GATGATGAAC	FIGACGACGA		CACCACCA	לאלביני של בינוני			-	TTTTGCACAA	AACCCTGG();
	CTACTACTTO	בישנישנים אל	ריאיר						CANCINCTING	ACCAGGCCAA
16301	GCACCACCG	AGICTITACO	CCCCTTCAGC		CACTTACANG				CHOCACCAAC	ALL CALL OF THE PARTY OF THE PA
	CGTOGTOOCA	TCAGANATGC	ROCCACTOS	CGACGTGGGC	CTCCGATGTTC	CCCCACATAC	TACTCCACAT		יופפורסאיר	ico ccom
16401	CONTRACT STATE	COSTONETTO	CCTACGGAAA	CCCCCATANG	CACATGCTGG	יניושייניניבים			CTAGCCTAAA	GCCCGTMACA
	Carry accepto	CCCCTCAAAC		COCCGTATTC	CTGTACGACC	GCANCOGCGA	ccrecreced	PICCEPICIO	GATCGGATTT	COCCENTRAL
	F.									Kpni
16501	CHARLED BORD	Practice Code	GCTTGCACCG	TCCGNACANA	AGCGCGGCCT	NANCETECTAG	THETOGETISACT		CONOCAGETO	ATCITACICCA
10001	GACGTCGTCC				TCCCGCCG3A	TFICACION	AGACCACTGA	ACCGROGOTO	GCACGTCGAC	TACCATURGET
16601	Brank Para			MATGACCGT	GGAACCTTAX	הדאמאתכככק	AGGTCCGCGT		ANGCAGGTGG	COCCGGGACT
10001	TCGCGGTCGC			THINCTEGEA	CCTTRIGACTC	GACCTCGGGC	TCCAGGGGA	COCCOOLITAG	TTCGTCCACC	GCGCCCCTGA
16701	Grandfille		TTCAGATACC	CACTACCAGE	AGCACCAGTA	THOCCACCGC	CACAGAGGGC	ATCCACACAC	MACGICCCC	COTTRICCTOR
10101	CreatAcon	_	-	-	TCCTCGTCAT	ANCIGOTGGCG	GIGICICCCG	TACCTICTIGITO	TTTOCAGGGG	CCAACGGAGT
10821					CGTCCANGAC	CTCTACTAR	GTICCAAACTIG		GTTICGCGTT	TCAGCCCCCC
	CENTRACORCE		٠.	_		GAGATACCITC	CACGITITICC	TOCCCACCTA	CARACCCCAA	AGTCGGGGG
10071				CCCCACCC	GCTACTGCCC	GANTATGCCC	TACATOCTTO	CATTGCGCCT	ACCCCCCGGCT	ATCGTGGCT
10201	STATE OF THE STATE		-			CTTATACGGG	ATCTAGGAAG	GTAACGCGGA	TOCOCOCCOA	TAGCACCGAT
•			_			GAACCCGCCG	CCTACATATACGC	CCTCGCCAGC	cconocitac	CCCGATTICC
1/001	CACCTACCGC	C CCCAGAMGAC						GCAGCGGTCG	GCCACGACCG	GCCCTAAAGG
	GIGGAIGGE						CCCARCATEG	TTTAAAAGCC	GGICTITICIO	GTTCTTGCAG
1/101	GTGCGCMGG							AAATTTTCGG	CCAGAAACAC	CAAGAACTGTY
	CACGCGTCCC	c Accoalocaci	r rechedence	. וואהארראני						Sptv
•		-	• تطفيد فقاماه الأرد	. המשומים	ATTICCOLOGA	AGANTGCACC		הדאמהאפסהם כאדפפככפסכ	CACGGCCTUA	בממכנושיבאיוי
107/1	TATACCCC						CATCCTCCC	GTACCOGCCG	GTGCCGGACT	OCCCGCCGTA
	50				Split					
17301	مبر	الا الدوالليانان	Contraction of	GTCGCACCGT	. כנא.עשואכנוכני	: OCOCTATCCT	GCCCCTCCTT		TOCOCOCOCO	
400.1	COCACACAC		פ ככפבכפנפכם		ACCITACGCGC	COCCATAGE	CCCCCCACCAA	TANGGIGACT	AGCGGCGCCCG	
17401			r case Procedo	S GCGCAGAGAC	: ACTIGATTAAA	A ANCIANTITUE	ATGTGGAAAA	ATCARABTAR	ANAGICTOGA	
1051					FCACTAATITE	r TREFFEACE	TACACCIPITY	TAGTITITATT	TTTCAGACCT	GAGACTGCGA
							!			
17501	COCPTOO	TCC TGTAACTATT	T TTCTAGAATG	3 GAAGACATCA	ACTITIOCGIC	GANGACATCA ACTITIGGGTC TICTGREECECT CGACACGGET	- ככאכאננמסכב	CACACCCGFF		
 	GCGMCC		ACATTGATAA AACATCTTAC	CONCIGINGT	T TGAMACGCAC	TCANACGCAG AGACCGTXXXC	GCTGTGCCGA	GCGCGGGCAA	Gracecrine	ACCOLLCIA

Figure 15K

	Emfl									
17501	THE PROPERTY AND	CARTATGAGE	GENGERAL	Tracercaso	CHUCKLAND	ACCERSCATTA	AAAATTTATG	TTCCACCGTT 1	ANGAÄCTATG	GCAGCAACAC
1	AGCCGTGGTC					TVOCCOTAAT	TITITANAGE !	ANGCTOCCAA '	TTCTTGATAC	cercerree:
17701	CTCCAACAGC	ACCACAGGCC TCGTGTCCGG	AGATYPCTYSMS TCTACGACTC	GGATAAGTTO	ξĖ	ATTTCCAACA TAAAGGTTGT	AAACCTCCTA (TTTCCACCAT	GATGGCCTGG (CTACCGGACC)	CCTCTGGCAT	TAGCGCCCA:
17801	GTOGACCTOG	CCAACCAGGC	AGTGCAAAAT	AAGATTAAGA	CATTESTANCT	TCCCGGCCT	CCCSTAGAGG	AGCCTCCACC TCGGAGGTGG	CCCCCTCCAC	ACAGTGICP: TGTCACAG7::
17901	CAGAGGGGCG	TOCCCAAAAG	CGPACACACAC	CCGACAGGGA	AGANACTOTIS TOTTTICACAC	CACTGCGTTT	TASACGAGCC	TCCCTCGTAC AGGGAGCATG	CACCACCGTO	TAMAGCANT: ATTICGITIC:
18001	CCTOCCCACC			TOCOCOCOT OCCTACTOGA AGCOCOCOTA CCGATOGCCT	הדנידידים כאכניאכניכנים	AGCACACACC TCC:TC:TC:TC:TGC	COTAACGCTG	GACCTCCCTC	CCCCCGCCGA GGGGCGGCT Prof	CACCC_NGCAV: GNCGCTCGTV:
18101	AAACCTOTOC	TOCCAGOCCC ACGGTCCGGG	CTGGCGGCAA	GTTGTAACCC CAACATTKRG	CACCTAGCCG CAGGATCGGC	CCCCACCTC	מכסמכסמכס	CCAGCISTICC	OCCUPACE THE COCTAGE COCTAGE ACC	COGCCCOTAG GCCCCCATC
18201	CCAGTGGCAA	CTGGCAAAGC	ACACTGAACA	GCATCGTGGG CGTAGCACCC	TCTOGGGTG AGACCCCCAC	CAATCCCTRA	Ancoccoard Trereserted	ATGCTTCTGA TACGAAGACT	TAGETAGET	GICCIAIGIN CACCATACA I
18301	TGTCATOTAT ACAGTACATA		CGCCGCCAGA	CETEGACGAC	AGGGGCGGGGG	CCCCCCCTTT	CCAAGATYBC	TACCCCTTCG ATGGGGAAGC	ATCATGCCGC TACTACGGCG	AGTOGICTI A TCACCAGA: r
18401	CATGCACATC			GTACCTGAGC CATGGACTCG	CCCCAGCTGG	TCCAGTTTGC ACCTCAAACG	CCGCGCCACC	GAGACGTACT	TCAGCCTGAA AGTCGGACTT	TANCAAGTIIT
18501	AGAACCCCA TCTTTGGGGT				ACCORDICICA TGGCCAGGGT	CCCTTTCACG CCCAAACTGC	CTGCGATTCA GACGCCAAGT	TCCCTGTGGA AGGGACACCT	CCCTGAGGAT	ACTOCOTACT TOACGCATGA
18601	COTACAAGGC	COCCANGTOD	CTACCTGTOG	CACTATIVOC	TGTGCTGGAC ACACGACCTG	ATGGCTTCCA TACCGAAGGT	CCTACTFTCA GCATGAAACT	CATCCOCOGC	GIGCTGGACA	OCCCCIOCATI
18701	THTTAAGCCC	TACTOTOGCA	CTGCCTACAA GACGGATGTT	CCCCCTCCCT	CCCANGRATIC	CCCCNAATCC GGGTTTAGG	TTGCGAATGG AACGCTTACC	CTACTTCGAC	CTACTGCTCT	TGAAATAAN ACTITATIIN:
18801	CTAGAAGAAG	ACCACCATCA TCCTCCTACT	CAACGAAGAC GTTGCTTCTG	GAAGTAGACG	AGCAACTGA TUGTTECACT	GUNGUAAAAA	ACTEACRETAT TRACITACATA	TTOGGCAGGC AACCCGTCCG	OCCITIATION COCRATARIA	GCTATAAATA CCATATTTAT
18901	TTACAAAGGA	00	ATAGGTUTCG	AAGGTCAAAC	ACCTAAATAT TGGATTTATA	GCCCATAAAA CGCCTATTTT	CATTHCAACC	TGAACCTCAA ACTTGGAGTT	ATACCACATA TATCCTCTTA	CTCAGTVGTA GAGTCAGCAT
19001	CCAAACAGAA	ATTAATCATO	CAGCTGGGAG	AGTCCTAAAA TCAGGATTTT	AAGACTACCC TTCTGATCCG	CAATGAAACC	ATT TTACAGE TACAATGCCA	TCATATGCAA AGTATACGTT	AACCCACAAA TTGGGTGTTT	TCATATACC"
19101	CCCGTTCCGT	TTCTTGTAAA	CONCONNT	GGAAAAGCTAG	AAAGTCAAGT TTTCAGTTCA	CCTTTACGIT	TITTICICAL	CTACTGAGGC	AGCCGCAGGC TCGGCGTCCG	AATGGTGAT · TTACCACTA r
19201	ACTITICACTICC TGAACTGAGG	TAAAGTGGTA	A THETACAGING		ANGATGTAGA TATMGAAACC TTCTACATCT ATATCTTTGG	CCACACACTC	ATATTICTTA CATGCCCACT TATAAAGAAT GTACGGGTGA	CATGCCCACT	ATTANGGANG TAATTCCTTC	GATTGAGTGE:

Figure 15L

19301	ACAACTAATIC	CCCCAACAAT	CTATCCCCAA	CAGGCCTANT	TACATTECTT .	TTATAGENTA	TTTTAPTCATE (COSTAATATG
•	TCTTGATTAC				ATTETAACGAA	ANTERCEMENT A	ANNATANCCA (GATTACATAA	remencence	CCCATTATAC
19401	GOTOTTOO						ACAGAGCTTT (CATACCAGCT	PHYCCHTGAT .	Tecatitions;
	CCACABGACC	GCCCGGFFCG	TAGGGTCAAC	TTACYINCAAC				-		A SCIENCE BAA
19501	ATAGMCCAG	GTACTITICS	ATCTICATANTC	ACKA: TOTTICA	-		-			AUB. T. I.C. MAN
٠	TATCTIGGIC	CATGAAAAGA	TACACCITING	TCCGACAACT	פדנימאדאנידא	GETCTACAAT (-			110000
19601	TIACTOCTIT	CCACTGGGAG	GTCTGATTAA	TACACACACT			-	-	-	TCCTACAGAA
•	AATGACOAAA	CONCOCCTC	CACACTAATT	ATCTCTGA	GAATYXTTTCC	ATTTOCATT .	TTOTCCAUTE (_		ACGATGICTT
19701	TITICAGATA	AAAATCAAAT	AAGAGTTCASA	AATAATTITIG	CCATCRAAAT	CANTETAANT	CCCAACCTIGT (-	CCHOTACTCC	AACATAGCO.
1	AAAAGTCTAT	THIRCTITA	THETCANCET	TTATTAMANC	GGTACCTTTA	GTTAGATTTA	COCITICGACA	CCTCTTTAAA	CCACATGAGG	TIGTATICGY: .
10801	Prof. A PPTICE	LTSACAAGCTA	AAGTACAGTC	CHECANCGE	AAAAATTTCT	GATMACCTAA	ACACCTACGA	CTACATGAAC	AAGCGAGTOG	TOGCTCCC(*:
1	ACATAAACGG	OCTOTICGAT	TTCATGTCAG	GAAGGTTGCA	ADMATTER	CTATTCGGTTT	TCTCCATCCT	CATCTACTTO	Treachean	ACCGAGGGGC
19901	CATTACHICAGAC	TOCTACATTA	ACCTIOGNAC	ACGCTAGITCC	CTTCACTATA	TOTACAACGT	CARCCCATT	AACCACCACC	GCAATGCTTGG	CCTGCGCTAC
1	CGATCACCTG	ACCATGTAAT	TEGAACCTCE	TOCGACCAGG	GAACTGATAT	ACCTIGITISCA	GITCOGTANA	rrecrestes	COTTACCACC	GCACCCCATG
20001	COLTYAANGE	TOCTOGGCAA	TGGTCGCTAT	GIGCCCTICC	ACATECACAT	CCCTCACAAG	TICTTIGGEA	TTAMANACCT	certescric	CCGGCTCAT
	GCGAGTTACA	ACGACCCGTT	ACCAGCGATA	CACGGGAAGG	TGTARGTCCA	COCACTUTO	NACHANCOCT	AATTTTTGGA	GGAAGAGGAC	GGCCCGAGTA
					Psti					
•		CHRONIA B. Cremen	ACCORDED TO	TTABCATICET	בו בוב שנישנים	TCCCTAGGNA	ATGACCTAAG	COTTCACCCA	CCCACCATTA	AGTTTGATA:
70107	TOTAL STATE	CACCHICAAG			AGACTICICG	AGGATCCTT	TACTGGATTC	CCAACTGCCT	COCTCCTAAT	TCANACTATIC
•			-		ACTORICA	CCCTTGAGGC	CATGCTTAGA	MACGACACCA	ACCACCACTIC	CITTAACG/
10202	CATTIOCCIT	Americanist	AGAAGGGGTA		TOXXCKGAGGT	GCGAACTCCG	GTACGAATCT	PROCEEDINGS	TECTEGRACAG	GAMPTOCT :
10101		_	GCTCTACCCT		ACCETACCAA	CONGCCCATA	TCCATCCCCT	CCCGCAACTG	GCCCCTTTC	COCCHOLOGIA
1000			CGACATOOGA	-	TEXCENTEGIT	GCACGGGTAT	ACCTACCCCA	GOCCOTTGAC	CCGCCGAAAG	GCGCCC3ACT.T.
10000		_	AAGGAAACCC	-	CTCCGGCTAC	GACCCTTAIT	ACACCTACTC	TOCCICTATA	CCCTACCTAG	ATGGAACTT"
	COMMETCIC	_	-	-	GAGCCCGATTS	CTGGGAATAA	TCTCCATCAG	ACCGAGATAT	GGGATGGATC	TACCTTCKICA
20501	TACCTCARC	CACACCTITA	AGANGUTGGC	CATTACCTITE	GACTCTTCTO	TCAGCTUREC	TRICCANTRIAC	CCCCTOCTTA	CCCCCAACGA	GITHAMATT
	AATOGAGTIG		-	GTAATGGAAA	CTCAGAAGAC	ACTECIACES	ACCGITACTO	GCCCACCAAT	COCCUTICET	CANACTITIAN
20601	AAGCGCTCAG	TTGACGGGGA	GGGTTACAAC	GTIGCCCAGT	CTAACATGAC	-	TTCCTYTCTAC	ANATOCTAGO		ATTK#XCFACK
•	TTCGCGAGTC			CAACGGGTCA	CATTIGITACTIC	CITTICITEMEC	AAGGACCATG	TTTACCATCG		TMCCGATGS
20701	MARKETICIA		ACCTACANGG	* ACCOCATOTA	CICCITICITY	AGAMCTICC	AGCCCATGAG	CCGTCAGGTG	GTGGATGATA	CTANATACAN
0	TCCCGAAGAT			TYGCGTACAT	GAGGAAGAAA	TCTTTCANCG	PCCCCTACTC	GCCACHCCAC	CACCTACTAT	CATTTATCTT
20801	COACTACCA	CAGGTGGGCA	TCCTACACCA	ACACANCANC	TCTOSATTES	TIEGETINCE	TOCCCCCACC	ATCCCCCAAG	GACAGGCCTA	CCCTCACTAM.
1	CCTCATOGIC		AGGATGTGGT	" TOTOTTOTIO	AGACCTANAC	AACCGATOGA	ACCCCCCCCTCC	TACGCGCTTC	CTGTCCGGAT	GOOMCGAITIC
							Peul			
20901	TRECCEPA	IC COCTTATAGG	CANGACCECA	I GTTGACAGCA	TTACCCAGAA	AAAGTTTTCTT	TEXTRACTORY			
i • •	AAGGGGATI			r concretencer	· AATGGGTTCTT	TITCAAAGAA	ACGCTAGCGT	GGGWWCCGC	GTACGCTAAG	ACCITCATION

Figure ISM

v	
r	
-	
_	
×	
÷	
Σ	
_	
_	
. .	
Ē.	
Ė	
האק	
÷	
Š	
÷	
Š	
Š	
かいり	
なるななない	
1	

21001	TTATGTCCAT	GOCCICACTC	ACAGACCTAGG TGTCTAGACC	GCCANANCCT	TCTCTACGUE AGAGATGUAS	AACTESTS CAST	ACCCCCTIACA TGCGCCATCT	CATCACTTTT	GAGGTURATE	CCATCCACGA
21101	GCCCACCCTT CGGGTGGGAA	CTTTATGTTT	TGTTTGAAGT ACAAACTTCA	CTTTRANCATO GAMCTICICAC	GTCCGTGTGC CAGGCACACTS	ACTIVATICACIA! A TUGITICACIOT	מהכמככסמאם מהכמככסמאם	ATCGAAACCG TAGCTTTGGC	TOTACCTOCO AC/ TXCACOC	CACGCCCTT .
21201	TCGGCCGCA	ACCCACAAC	ATAMAGAAGC	MOCMCATC	AACAACAGCT TTGTTGTCGA	GCCGCCATGG	GCTCCMSTGA	GCAGGAACTG CGTCCTTGAC	AAAGCCATTG TTTCGGTAAC	TCAAAGATCT AGTFTCTAGA
21301	TOGPTICTODO ACCAACACCC	CCATATETT	TECGCACCTA ACCCCTGGAT	TYACAAGCGC ACTGTTCGCG	TTTCC AGOCT AAAGCTCCCA	THETTTCTCC	ACACAAGCTC TSTGTTCGAG	COGACOCOGT.	TAGTCAATAC ATCAGTTATG	GGCCGGTCT: C
21401	DAGACTODOS CTCTGACCCC	COCATGTGAC	CTACCGGAAA	GCCTFSGAACC	CGCACTCAAA	AACATOCTAC TTGTACCATG	CTCTTTGAGE GREANACTEG	CCTTTCCCTT	TTCTGACCAO AAGACTGGTC	CCACTCAAGC GCTGAGTTC1
21501	AGOTTFACCA TCCAAATGGT	CANACTCATO	GAGTCACTCC	TOCCCCCTAG ACCCCCCATC	CCCCATTCCT GCCCTAACGA	TCTTCCCCCG	ACCCCTGTAT TOCCGACATA	AACOCTGGAA	AAGTECACCC TTCAGGTGGG	AAAGCGTAC . TTTCGCATGT
21601	GOOGCCAAC CCCCGGGTTG	-	GICCACTAIT	CTGCTGCATG GACGACGTAC	TTTCTCCACG AAAGAGGTGC	CCTTTCCCAA	CTGGCCCCAA	ACTCCCATGG TGAGGGTACC	ATCACAACCC TAGTGTTGGG	CACCATGAN' GTGGTACTT
21701	CITATIACCO Gaataatggc	GOOTACCCAA	CTCCATGCTC GAGGTACGAG	AACAGTCCCC TTGTCAGGGG	AGGTACAGCC TCCATCFCGG	CACCCTGGGT	COCANCCAGO	AACAGCTCFA	CAGCTTCCTO	GAGCGCCAC" CTCGCGGTG
21801	COCCCTACTT	CCCCAGCCAC	AGTGCGCAGA TCACGCGTCT	TTAGGAGCGC AATCCTCGCG	CACTICITIT	TOTCACTTGA ACAGTGAACT	ANANCATGTA TITIGTACAT	AAAATAATGT TTTTATTACA	ACTAGAGACACA TGATC ICTGT	CTTTCAATAA Gaaagttaa-1
21901	AGGCAAATGC TCCGTTTACG	TTTTATTTGT	ACACTCTCGG TGTGAGAGCC	CACTAATAAA	ACCCCCACCC INSCRINGINGS	THGCCGTCTG AACGCCAGAC	CCCCCTTTAA	ANTCANGO	GCTTCTGCCG CCAAGACGGC	COCATCOCTA
22001	TOCOCCACTO ACOCOGTICAC	CONCECTION	GTTGCCATAC CAACGCTATG	TOGTGTTTAG ACCACAMATC	TYCTCCACTT ACTAGGTGAA EenHV	AAACTCAGGG TTTGAGTCCG	ACAACCATCC TGTTGGTAGO	GCGGCAGCTC	OGTGAAGTTT CCACTTCAAA	TCACTCCAC/. AGTGAGGTGT
22101	GOCTGCGCAC CCGACGCGTG	CATCACCAAC GTAGTGGTTG	GCCTTTACCA CGCAAATCGT	CCAGCCCCCC	CCATATCTTG	AAGTCGCAGT TTCAGCGTCA	TOGGGCCTCC ACCCCGGAGG	GCCCTGCGCG CGGGACGCGC	CCCCAOTTOC	GATACACAGG CTATGTGTCT
22201	GTTGCAGCAC	TOGAACACTA	TCAGCGCCCGG AGTCGCGGCC	GTGGTGCACG CACCACGTGC	CTYXCCAGCA	CCCACANCAG	CCTCTAGATCAGA	TCCGCGTCCA AGGCGCAGGT	GOTCCTCCGC	GTTGCTCAGG CAACGAGTCC
22301	GCGAACGGAG CGCTTGCCTC	TCAACTITIGG	TAGCTGCCTT ATCGACGGAA	CCCAAAAAGG	COCCOCACGOG	AGGCTTTGAG TCCGAAACTC	TTGCACTCTC AACGTGAGCG	ACCOTAGTOS TOGCATCACC	CATCAAAAGG	TGACCGTCCC ACTCCCACCC
22401	COCACACCC	GTTAGGATAC	ACCCCTRCA TCCCCRACCT	TAMAMGCCTT	GATCTGCTTA CTAGACGAAT	AAAGCCACCT	CTCGGAAACG	GCCTTCASAG CGGAAG I: TC	AAGAACATGC	CGFAAGACTFT
22501	GCCGGAAAAC		SII TGATTGGCCG GACAGGCCGC ACTAACCGGC CTGTCCGGCG	GTCGTGCACG CAGCACGTGC	CAGCACCTTG	CCTCCCTCTT	CCTCTAGACG	ACCACATITIC TOCTETAAAG	GGCCCCACCG CCGCGGTGGC	GTTCTTCACG

Attactacest Tacgnaggen	CNANCHER	CCAGGICTI-3 GFICCAGAAC	CCCCCTCC 1	CCTCTTCCG? GGAGAACCCA	GCTCIANACOC CGACTTTC 73	PAGADANGN	CCTCCTCGGA	ACCTCGCGC.	TCAGTCCAC:A AGTCAGCTC'	CCCCCCTTGA	AAAAGCAAGA CCAGFACANI TITICGINCE GGICCIGING Psil	CAGCGCCAGT		CTATCACATE GATACTETA; ECMIV	CCTUNTATY: GGACTATAR?
atttatcata taaatagtat	GTCACCTCTG	CCTCGTTCAG GGAGCAAGTC	CATCAGCGGG GTAGTCGCGC	TCTTCCTCTT AGAAGGAGAA	CCOGTGGGTT	AGAAGGGCGC TCTTCCCGCG	GACTECTTCCT	10GT1COOOD	GATCATOGAG	GTCGAGGCAC	AAAAGCAAGA TTTTCCTTCP P	GAAGCATCTG	CACCTATICT	TCCTTCCCAC ACGAACGCTG	CGCTGTCATA GCGACAGTAT
CONCENCENT I	ATCCTTCTAG TACGAACATC	CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC	CCATGAACAG	TTCGCTCGGC AAGCGACCCG	TICATTAGCA AACTAATCGT	CCCCCAACCC	GICTIGIGAT	ACGTCCTCCA TCCAGGAGGT	CCGTCTTTTT		ACAGAGGATA	ACCTOCTOTT TOCACGACAA	CTACGAACGC GATGCTTGCG	GTGCCAGAGG	CAGCHOOCUP TROMSCAGGO GTCGACTGGA ACRECCITECE
ATTTCAATCA TAAAGITTAGT	TGGGCTCGTG ACCCGAGGAC	CAGCINGDAAC	TTATCCACGT AATAGGTGCA	CACTITICCGC	TTTCCCATGC AAACGGTACG	GCCCCCCCGA	GCACCAGGGG	CERTACACCAC	TTCTCCTATA ANGAGGATAT		CTCAGTACCA	GTGGGAGACG CACCCTCTGC	TCAGCCTTRG AGTCGGAACO	CCTATTACC GCATAAACOG	
CCACTGTAGG CCACTGTAGG	GCGCAGCCPG CCGCGTCGGGC	TCCACTTCCA	CTTTAGATCG GAAATCTAGC	ACCGTANTIT	GCTTACCTCC CGAATCGACG	CTCTCTCTGAT	CCACACGCGC	GCGACGGGGA CGCTGCCCCT	GRECEATTICE CROSTANANGO	•	ACGAGGACCG TGCTCCTGGC	CTACCTAGAT	ATAGCGGATT	ACTINCTACCC TGAAGATGAG	AGCGGACAAG TCGCCTGTTC
CCTTTTTCCT		GTCTTRITTGC	TYSAAGTTYGGC ACTTYAAGGG	CGCGTTCATK: GCCCAAGTAG	rgcactrates ocgreacacg	CCACCATTAC GGTGCTAATG	CCCCCCCCTC	CCTCCGCCGC	CTTCCCCACT GAAGGGCTGA	CGAY,TCCACC GCGAAGATHAG	AGCGAAGACG TCGCFTCTGC	COCATOGICA	CONTRACTOR CONTRACTOR	CCCCCCCCCC	ACCCAGCCG
מנושנעליות במכמופענעט	CECTCCCCAC CRYXTHETTG	CONCACAAAG CITCINGITRIC GCAGIGITIIC CAGAACAACG	CCCACTAGTT	GCACACTCAG	ATTCAGCCGC TAAGTCGGCG	TCCTCCCTCT AGGACTGACA	ACCITCTATED TCCAGCTACC	حددودعصود	COCTOCTCCT	TCGCCACCAC ACCGCTCGTG	AGGITITIGIA TCCANAACAT	CCCCTCCTT	GCAGCGATGT	CCAGCCCAAC	TOCCONTOCON ACCOCACGOT
כחנירדונים האדמערנאת	TCGATCTCAG AGCTAGAGTC	GCCCCATCAT CGCCCATCATA	CACTTROTTCA GTGAACCAGT Fred	GACACGATCG CTCTGCTAGC	CCAGCAGAAG	TACTCTTTCT AAGAGAAAGA	TCCGCCGCCG		CCACCAMOC		ACCACCACCC TCGTCCTCCC	AGTETAGEOGG			F ACCCCTATCC
TRETAGACTR	AAGCTCGCCT TTCGAGCGGA Pstl	TOCAGGAATC	CCAGAGCTTC GGTCTCGAAG	CTCCCACGCA	CICCOCCACTO	GCGCCACATC	ANTOGCCAAA	COCCTCATCC			CACTAATAGE	ACCACCAACA			A ACTOCAAGAT
ATCTTGGCCT TAGAACCGGA	GTAGACACTT CATCTGTGAA PVI	CAGGTACGCC	CATACGGCCG	CCATCCCCTT	CCCCATACCA	ACCATTTGTA TOOTAAACAT	TCTTGGGCGC	CTCCATACGC	CCACCGCGTC	AGAAGGACAG	CCTCCTCCTT	CCACAGGCAA	GCCCATTAT	ACCCCCAAA	TITITICCAAA AAAAAGGTTT
22601	22701	22801	22901	23001	23101	23201	23301	23401	23501	23601	23701	23801	23901	24001	24101

Figure 150

PMRKAdSgag MFR6R2

24201	CCTCGCTCAA	CGAAGTCCCA	MAATCTITG	ARKSTETTER	ACGENIACEME	AASTRICTER	CAAACCCTCT	CICANCAGGAA	ANCAGCGANA	ATGAMACTICA
	GCAGCCAGTT	GCTTCACGGT	TITTAGAAAC	TCCCAGAACC	TREACTECTE	TRUCKUCK	GITTIGCCAGA	CCTTGTCCTT	PPGTCCCTTF	TACTTTCAGE
		ŧ	Xhol							
24301	cretecagte	THEOTOCAAC	Techescrica	CAACGCCCCTTC	CTAGCCCTAC	TAMANTEAG	CATCGAGGTC	ACCCACTITO	CCTACCCGGC	ACTIMACCTA
	GAGACCTICAC	AACCACCTTO	AGCTCCCACT	GTTGCGCGCG	GATCGGCATC	ATTTICKOTO	GTACKTECAG	TIXXTICAAAC	GCATGGGCCG	TGANTTGGAT
24401	CCCCCCAAGG	TCATCACCAC	AGTCATGAGT	GARCTCATCG	THICHTCOINE	ההאהריבידה	CACAGGGGATG	CAAATTTGCA	AGANCAAACA	CAGGAGGG"
	GGGGGGTTCC	AGTACTCGTG	TCAGTACTCA	CTCGACTAGC	ACKINGGEACG	CATHOGRAPHIC	CTCTCCCTAC	GTTTAAACGT	terrenter	CTCCTCCCC
24501	TACCCCCCAGT	TOCCCACGAG	CACCTAGGG	GCTCACTTCA	AACGCCCCACA	CCTCCCCACT	TGGAGGCG	ACCCAAACTA	ATGATGGCCG	CAGTV:CTCTT
	ATGGGCGTCA	Acceptedic	CITCGATCCCC	CCACCGAAGT	TTYSCACCOCTIC	GGACGGCTGA	ACCTCCTCCC	TOCGTITICAL	TACTACCODE	GTCACGAGCA
		S AND	open The second							•
24601	TACCGTGGAG	CTTCAGTGCA	TGCAGCGGTT	CTTTGCTCAC	CCGGAGATAC	AGCGCAAGCT	ACACHANACA	TRECACTACA	CCTITICGACA	COCCTACGTA
	ATOOCACCTC	CHARCTCACGT	ACGICGCCAA	GAMCGACTG	GGCCTCTACG	TOGOGITTOGA	Terechings	AACCTGATGE	CCAMACCTCT	CCCGATCCAT
		Bott								•
24701	COCCAGOCCT	GCAAGATCTC	CAACCTGGAG	CTCTCCAACC	TOGSTOTECTA	CCTTGGAATT	TTGCACGAAA	ACCGCCTT00	GCANACGTG	CTTCATTCCA
	GCGCTCCGGA	COFFICTAGAG	OFFICEACCIC	GAGACOTIOG	ACCNGNGGAT	CCAACCTTAA	MCGIOCITIT	TOCCOGNACC	COTTITICCAC	CAACTAACOT
		Asc	ł							
24801	CGCTCAAGOG	CGAGGCGCGC	COCCACTACO	TCCGCGACTG	CCTTTACTTA	TPICTATGET	ACACCTIGGCA	GACGCCCATG	GCCOTTTGGC	AGCAGTUCT
	GCGAGTTCCC	GCTCCGCGCG	GCGCTGATGC	AGGCGCTGAC	GCMATGAAT	AAAGATACGA	TETCHARACCGT	CTGCCGGTAC	CCGCAAACCG	TEGTCACGAA
			PSII							
24901	OCADGAGTOC	AACCTCAAGG	AGCTGCAGA	ACTOCITAMAG	CNAMICTICA	AGGACCTATG	GACGGCCTTC	AACGAGCGCF	CCCTGGCCCCC	OCACCTOSIC:
	CCTCCTCACO	TICGAGILICC	PCGACOTICIT	TCACCATTTC	GTTTTGAACT	TCCTGGATAC	CTOUCHGANG	TYBCTYCGCGA	GGCACCOGCG	conserved:
25001	GACATCATIT	TECCEGAACO	CCTGCTTANA	ACCUTOCAAC	ARKSTOTOCC	AGACTITCACC	AGTCANAGCA	TOTTGCAGAA	CTTTAGGAAC	PTTATCCTN'
	CTOTAGTABA	AGGGGCTTGC	GGACCAANTIT	TGGGACGTTG	TCCCAGACGG	TCTGAAGTGG	TCAGITTICGE	ACAACGICIT	GANATCCTTG	AAATAGGATI'
25101	ACCUCITCAGG	AATCTTGCCC	GCCACCTOCT	GTGCACTTCC	TAGCCACTT	GTGCCCAPTA	ACTACCCCCA	ATGCCCTCCG	CCCCTTTGGG	GCCACTGCTA
	TCGCGAGTCC	TTAGAACGGG	COGTOCACCA	CACGTGAAGG	ATCCCTGAAA	CACGGGTAAT	TCATGGCGCT	TACOGGAGGC	GGCGAAACCC	COGTGACGAT
	Patl									
25201	CCTICTGCNG	CTAGCCAACT	ACCITIOCCIA	CCACTCTGAC	ATAATGGAAG	ACCINGAGGGG	TIGACGGTCTA	CTOGAGNETIC	ACTOTOGCTO	CAACCTATCC
	GCAAGACGTC	GATCGGTTGA	TOGANCOGAT	GGTGAGACTG	TATTACCITC	TOCACTOCCC	ACTOCCAGAT	GACCTCACAG	TGACAGCGAC	GITGGATACT
						<u> </u>	101 2000	Pstl		
25301	ACCCCGCACC	GCTCCCTGGT	TTGCAATTCG	CACCTCCTTA	ACCIANAGEICA	AATTATCGG	ACCTITICAGE	TECAGGGTCC	CTCGCCTGAC	GANAGICCA
٠	TOCOCOTOO	CGAGGGACCA	AACGITAAGC	GTCGACGAAT	TOCTTTCAGT	TTAATAGCCA	TOGRAACTCG	ACGTCCCNGG	GAGCGGACTG	CHITTICAGG.
25401	COGCICCOOC	GTTGAMCTC	ACTCCGGGGC	TOTOGACGTC	GGCTTACCTT	CCCAAATTTC	TACCTIGACICA	CTACCACGCC	CACGAGATTA	GOTTCTACGA
	GCCCAGGCCC	CAACTTTICAG	TCAGGCCCCCG	ACACCTGCAG	CCGAATGGAA	GCGTTTNAC	ATGGACTCCT	CATECTOCOG	GTGCTCTAAT	CCMAGATUTT
25501	AGACCAATCC	COCCCOCCTA	ATGCGGAGCT	TACCRECTASC	CHIATTACCC	NATIONALIAN	TCTTROGCCAA	TTGCAAGCCA	TCAACAAAGC	CCCCCANGA
	TCTOGTTAGG	GCCCCCCCAT	TACGCCTCGA	ATGGCGGALIG	CANTAATYGG	Trechanter	ACAACCOGIT	AACGTTCGGT	AGITICITICG	GOCKETTICH.
25601	TITCHGCTAC	GANAGOGACO	GGGGGTTTAC	THOCACCCC	AGTCCCGGTGA	OGAGCTCANC	CCANTCCCCC	CUCCUCCUCCACA	GCCCTATCAG	CARCARRER
	BABCACCAN			AACTERVENING	TURNISCOND		Catalland		CONCAPACION	

Figure 15P

CENCHOCOCCA

CCTACCCAGA TGAAATTGCG GAAGAGCCCT

CCATCOGICT ACTITAACCC

GGAACTCTCC ANTITATTICA CGAGITTICTO

AGACOTICTOS AGCAGGAGAC TUGOCGUGAG AUCTUUGTAA CUTTGAGAUG TTAAATAACT

TOGAGGCATT

POSTCOTOTO ACCOCOCOTO

TCTGCAGACC

27201

CCTCANACAC

CAGCTCAACT

GCCAGGTATT

CANTCAGAGG GTTAGTCTCC THICATHEREGG

ACTICACCTGA

CCCCCCCCCCC

CCCTCCATAA CCTCGTCAGG

CTACTCATTG

GATCIACTIAN

TAPTGGAAT

ATMACCITTAN

CANTCCTIMC

GGAGCAGTCC

CTUGAGGAGE GAACEAGAGE CAGGECTICEE CTGTAAAGTE TAGECTACCGE GGECGGCGAG AAGTAAGTGE

GACATTICAG ATCGGCGGCG

GTCCGGACGG

CHIOCICICC

ACCIMOTOCOGY TECTICACICA

27101

GOOGNANDER GTGTCCCACG CCACACGCAC CCTCCCATAT TRAGTGGACT

COSCIPINGE CACAGOTING CONTENCORD GCAGGGTATA

ACCTTOCOCO GAGCTCCTCG

TCAGOGGCCC

27001

AGTECECCOCO

TEGAACTOCCC

CHICCHCCA

CICCOLACC: A TOTALCEACCE ACCOCCTGG CASCINCATION **GCGAGTACC**() TOTAL PROPERTY ACCHINARCO CHECHACOGGA CCGCTGGCCT

DMRKAd5gag MER682

25701

25801

25901

26001

26101

26301

26201

COCACCITADA ARGANICTRIC NATITATIVIR GACIORGIARIA ANTACTICADA CAGICAGGICA GAGIANATITA CCGAAGTTCA **GTCACTCCGT** NACAACAGCG CCAAGGCTAC ACCCCCACA CAGAAGCANA AACCCGTATC TCAGCGCCAT TATTACCACC ACACCTCOPA GALACACCOTT CTTTGTGGCA CACTGCCCGT GTGACGGCA **GGTTCCGATG** TUTTOTOTAC CATCACGGCG GTAGTGCCGC **SECTIONAL** TTCCCCATAG CACCICITO GTCCAGAGAC TOCCOCKE TO A ACCCCCCACT ACTCGCCCCTA AATAAACTAC TTATTTGATG TOTOGAGCAT CACCICAATG GROSSIGTIT ACCCIGAACG CCGACCTCGA CGGGITCTGA TGAGTTGGGC COCTITION ACAGINADO TOCCOCIÓN GOCCOCOO CONOGRACARO ACANGAGATO **NANTRAMAN** ATAATGGTGG GACGCCCAGG CTGCGGGTCC CACTAAATAC TAGINGAAGE CGCTHXCHAC CTTCTGCGCC TCCGAGAGAA GTCATTTATG **GCACCTGTTG** TTATGACCCT GETGTCAGAC CCACAGICTG 0000000000 CACCOCCCCC THEFTGICGC recedence GCCCCCAACG COCOSOTICC TITATITI ACTICAACCCG GTCAACGGAA TACGCGCCCA CCCAAACCGA ATTITICITIG AACAGGCGGC THETECOCCG ACTITICOLAGA TCAACCETCT ACTERRARAGE CETANACEAGE GANGETTCCG AGGTCGAAGA TCCAGCTTCT GREANERGET TREARCATES CTACANCETE CRETERITORS GCCGGTANGT CCAMICAGC GCCGCGGTTA GCCCANGAGC CGACGCAGAC CANGACCTOA CCCCCCCCCA **OCCCANGACT** CTISCTCCTTCC GATESTEGRAC GCGAGGAGTC CCCCTTCTCC CCCIXCCICT CAACGAACGA ACGITCTGAC ACCCCCGTTG TAGAAGAAGC GGGGGGGAA CAGGNACAGG Greenwerce GCTCCCGTCTC GTTCTCGACT AGCTCTCTT GOGACCACAT GETCCTTTCA GAGCGAGGT GATGACACCA CTATAGOGCC CAGTICCCTT ATGCGCGGGT GACTITICGCT TAAGAGGACC CCCCCTCCCA CCACTGTGGT CCATATAMAG THORSTOTTC CCCGGTTCTT GCGANANCTA CRITCATCTCC ARCGRICCACA CONTRACTOR GSTPCGTCGG CGGCGGCAAT TRODGICCAAC ATCTCCTTCG TACTOCACOG GCCCCACAGCCG GATGTCOGGT ATGACGTYGC CGCCGTCGCC פתפתככככ כפונפתכפוכ כיוכנתכיוכם AACAGAGCAG GGGCCAAGAA ATCARCITICG GCGCACTCTTG GAAGACGCGG OTOGAGITAC CAGCCACAAA TGGCACTTCC GGCTGGAGCT CTTCGAAGGC GCARCAGCAG GAGGARAAGC CECESSANCE ANGOSTECTA CEGIGIATITA ITETTEGACG ITCACERCE GGATIC TICCTIC: GOOTETTING COGTINGCON AGRICUTING CCCTGGTGTA CCAGGAAAGT CACAGCGGCG TGACCCTTCTC CCCTIGATES ACCTINISTIC CONCENTION TOCANGACTO CTACAGCCCA CCTATATTIC TITABATTCG TOGANCCAGG TGCCAGTAGA **OCTIVETITAG** CACTOTOTAL GTGAGACATA THITCGCFFC **NATITIAN**CC CCAAGAAATC ANACCGNAG ACCORCATET COACCACCAC ATCATCOAC TACTACCTTIC CCCAGANATC GTTCCTTGCT ACCOMICGAC CFTCCCCTAT CTGCATTACT GACCITAATGA CTGACAAAGC GACTOTITICG GUATITITICS CCTANAAAGG CCTOTATCAC GCACATAGTO OCCUPIENCIE COCCANAGAG COCCCTACAT OCCOMATGTA GATATCCCGG TOCCCCCCTG GAACGCCATA TACCCAGGAT CCTCCTCCTG ACCCCCCCCC GOGACACCAC TCGCCGGCCC EDOFIV GALATTECECA CHOCOCOTICTA **TCCCCGFAGT** CTTAGAAACA CCCCCACCTG COCCURCGAC CTAGTTTCGC GATCANAGCG CITTAAGGGT DACCECACAT GOOGCATICA ATCOTTCTGA GAATCTTTGT TOOCATCTA **OCCORDICACAA GCCATTICTAG** TAGCAAGACT **GCCTAAGGGG** COCCONOTT CCCTAACATC DOCCCTTOC ACCTGCTCCT CGCATTCCCC AACCGFAGAT TOCACCACCA 26801 26901 26401 26501 26601

CCATCCCTCA

CCTACOCACT CTCTTAAGGA

CACCCCCCCAG

CTGGGGGGGTTC

TATGRECAM

GAUAATTCC

ATACTICGTTC

ATGACCCCCG

TACTCGCGC

Figure 150

26701

GCAGAGCAAC COTCTCC1TF3	ATATCCAGCIC TATAGCTCCT	GGACAGGG IA	AGNANTIA:A		-		3 CTATTICTANT C GATAAGAT	-	C OTCAGNOCAC G CAGTOGGGW	C ACCACAGN (G TGGTGTCT); G TTACAGTTI'I C AATGTCAAAA	00	A TACANAAGCA. T ATGITTTCC C	
AAGTOCAGAG TTCACCTCTC	CCCCACACATC GGGCTCCTAG	TAGTTGAGCG ATCAACTCGC	TAATAAATAC ATTATTTATG	TACTTTTAAC ATGAAAATTO	AACACCACCC TTGTGGTGGG	TCAATAACTC AGTTATTGAG	ACTICTACOOG TGAGATGCCC	AAGACTCGCC	TCACCCTTGC AGTGGGAACG	TATAAAATGC ATATTTTACG GAGTATAATG	agtataagtt Tcatattcaa	CTATATTAAA GATATAATTT	DACGAACGTT TCTATGTGGG AGATACACC
ACTGAATGTT TTACTA	CITHGANTIO GNAACTTIANC	CCCCCCTCC	GRECTCHOTA CACCACTCAT	CCTTACCTOO	CATCAGAAAA	COCACAGACC	AATTCAAGCA TTAAGTTCGT	•	CTACCTTTAC	CCACCACTCT CCTGGTGAGA TGACACTACA			CCAAATGAGC AACAATGAGC TTGTTAACTG
GACGGCTACG CTGCCGATGC	ACTITITICCTA TCAAAACGAT	GTTTACCCAG CAAATGGGTC	TGCCATCTCT ACGGTAGAGA	CCANGCCGAA	TCAGCTACTC AGTCGATGAG	AGACTTTTTC TCTGAAAAAG	GTTTATGAAC CAAATACTTG	ATACTARCOC TATGATTOCG	GTACATAATC CATGTATTAG	GCTANTGAGT CGATTACTCA CGCAGCCAGG			ACCACTANCT TOGICATICA CATTCCCCTO GTAAGGGGAC
המאנידנים המאנידנים המאנידנים	CHEAGGCCAC	GCCT	AGATCTTTGT TCTAGAAACA	CCCAACCAAA GGGTTCGTTT	CTCTCCCAGC GAGAGGCTCG	ACCGTANACC TCGCATTTGG	CTACTGTGTG GATGACACCC	CTTTATTCTT GANTAAGAA	MGATGATTAG TCTACTAATC	CGCAGCTGAA GCGTCGACTT TATGCTATTT	TGTGCGACAT	TACAGTECTE	AGCTAATGE TCGATTACAG TCCTCAATAC ACGAGTTATG
ACCATACTANA C	CHAMICITAL CANALOGUE		GATTACATCA	TCTTCACCCG AGANGTGGGC	ACCIACIAGAAC TOCTCTCTTG	CTACCGCCTG	AAAAGCGCAG TTTCCGCGTC	THETGATTET	TCGCCACCCA AGCGCTGGGT	ATGITACATT TACAATGIAA GTATGICIGIT	THITATGAM	CTATCCTAAT	TANGTTACAA ATTCAATGTT GGTCATTTCC CCAGTAAAGG
CCTANCITING A	CCCACAACTIC C	-	CCTAACCCTG (CTCACTCAGA	ACGTOCTOTO	GTATTAGGCC	ATTCTCTGTC	AACGCTRICKO TTGCGACCCC	CCAGCCTGTA GGTCGCACAT AAATTGGCAA	ION TACTITICCA ATGANAGGT	TOCTOCACTO ACGACGTGAC	CTTAATTTAC GAATTAAATG TAAACCCCCC
TCAATMTATT C	CACTGTCCCC C	-	TTGCAACTGT			CACCOCCCC	AACCCTTAGG TTGGGAATCC	OCTROGGETT ECAACCCCAA	CACCTITITIA	AAAATTCCTC CACAAAACA	ESHION ESHION CHTTATGRA TACTITICGA GAAAATACAT ATGANAAGGT	TOGCACTTTC ACCGTGAAAG	AAGAAAATGC TYCTTTTACG GAATAGGAFF CTTATCCTAA
ACTATCCOGA 1		-	TCACTOTGAT		CAACAGTTTC	ACCAGICCOT	GAGCTTAGAA	CTAGAATOGO GATCTTAGCC	CATPTATTOT GIAAATAACA	AAAAGGTGGA TETTCCACCT GCTTATTCGC	CUANTANGEO AGTCATAMA TCAGTATTTT	TOGAMACAC ACCTTTTGTO	TATTGAGGAA ATAACTCCTT ATTATAATTA
CCTCCCGGCC A	-		CCCTOTOTIC			CCGGGAACGT	AACAGGAGGT THGTCCTCCA	TCAGGITTCT	TOCACATTIG	Kori GGTACCACC CCATGGTGGG ATGAAAAGCT	CCAGGGTAAA GGTCCCATTT	CAAATTGTG	GACGCAGCTT CTCCGTCGAA AAAAGTTAGC
27301	27401	27501	27601	27701	27801	27901	28001	28101	28201	28301	28501	28601	28701

PROMOCRET CCARCTACAG CONCCACTE ANCANOSICA GOTTOATOTE GCTIXXIII	ACTITICIDEC TITIGICANIA ACTICANIA TOANGACGE AAACAGTTAT TOANCCTATIT	CTAAAGCCCA AACOGGCCGC ACCACCCATC GATTACGCGT TTGCGCGGCC TOGTVX7:TAG	TCTTTTCTCT TACAGTATCA TTANATCAVA ACAAAAGAGA ATGTCATACT AATTTACTCT	GTTTCTCACA TCGAAGTAGA CTGCATTC A CAAAGAGTGT AGCTTCATCT GACGTANG T	TCGCCTTTAT CCARTGCATT GACTGGGT.T AGCGGAATA GGTCACGTAA CTGACCCAGA	TTCTTAGAAT TCTTTAATTA TGANATTTAC TGTGACTTTTTAAATG ACACTGAAAA	GATTCACTCG TATATGGAAT ATTCCAAGIT CTAAGTGAGC ATATACCTTA TAAGGTTCAA	TACCANCITA GCCCTAGCTA TATANCCCIA	GACGTIOTIC ACAACGCC CCCAAAC XBBI Bgft	ATGACTGACA CCCTA	S ACCITICANDA CATOGITAAC TIGGACGAGT. TUGAGGITICI GIACCAATIG AACHIGGICA	CCTTAGCTAC	ATTURCTOR CTITUTORIANS TRACTORION GRACAGITUCE	MAMAMATAA TAAAGCATCA CITACITAAA ATCAGITASC TITITITATT ATTICGIAGI GAATGAATIT TAGICAATCG
TCCCGCGCGAT AGGCGCGCTA	ATACACCCCA TATGINGGGT	CATICTOCTEC	AAACACATGE TETGEGEACA	ATTGGCTGCG T/MCCGACGC	ACTISTOCTICA TGACACCAGT EGOF	TTCTTAGAAT	ATATCATGCA TATAGTACGT Psil	TGTTCTGCAG ACAAGACGTC	TATACGAAGGT	ACAGGAGAGAG TGTCCTCCTC	ATGAATCAAG TACTTAGTTC		GCTTCCGACG	
CCAGCACCTG	TCTACCACAA AGATEKTICTT	TTATCH(X)CT	CENCECNOTO CENGRAC	CGTGCTCCAC GCACGACGTG	CAGCCTCATC GTCCXIAGTAG	ATAGCTGAGC TATCGACTCG	TCAAAGACAF AGTTTCTGTA	TCTGTTATGG AGACAATACC	CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC	CTTTAATCTA GAAATTAGAT	CCAACAGCGC CGTTGTCGCG	AGTANTACCA TCATTATGGT	GCCATCITIG	TAACTAATAA ATTGATTATT
CHGACHTHUG G	CCCACTTACA CCCCTCAAATCT	ACGUATANT	TCCATAGATT	CTTTTTTTTTTT CGTSCTCCAC GAAAAACAC GCACGACTTG	CACTENTETA CACATICATE GEGAGTAGAE GILEGAGTAG	GGACAGGACT	CCTCCAAGCC	TCCAATCATC ACCTTAGTAG	CCANCITICC GGITGAAAGG	AAATCAGCTA	CAGCGGCCGA		ACTCAGCACT TGAGTCGTGA	CTCAAAGATC TFATTCCCTT GAGTTTCTAG AATAAGGAA
ATCHCAGEAT C	בכבכבהרואר כ מכבבבהאוה	TATESTATES I	AATCATETAA	CCTTGTTGCG (CAGTGGGAGT	CCCAGTACAG	TGTTCCCCGA	CCTGGTTATA GGACCAATAT	Catgaaccac Gtacttegtg	ACCYCCACTG TGGGGGTGAC	AGACGCAGGG TCTGCGTCCC	AGGCCAAAGT TCCGGTTTCA	CATTACCATA GTAATGGTAT Bedi	
GGCTTCCTR96 /	ACCAMENCES O	CCATAGCGCT GGTATCGCGA	ACACCCAAAC TOTOGOCTTTG	TATTACTGAC	TTACGGATTT	AGACACCATC	ATCTGCGTTT	CTTTCCGAAG	CAATAGATGC GTTATCTACG	ACCIPICACC TOGANGAGGG	CCTGCTAGAA	CTCGTAAAGC	CACATATACCC	CCTGTGCGGT
CTTGAAGTCA C	GACCAACACA 7	TOGTOCTTOT C		CCAGTTTTTA .	TCTATTTGCT .	TOCATATOTO		AAAAAGCGAT	GOCTOGAACO	AGCCTCGCCC TCGGAGCGGG	CAGAGCAGCO	TATCTTTTGT	GICATOCTCC	TTATTANGAC AATAATTCTG
GCGCTACAAC C				×}	CCCTTCACAG	CACACGCGAA	< t	CCTACAATGA	CCTTGACAIT	CCAGCCANTC	CCITAATAAT	COMMOSOS	GAAATTOOTO	CTCTGCACCC GAGACGTGGG
28901	29001	29101	29201	29301	29401	29501	29601	29701	29801	29901	30001	30101	30201	30301

pMRKAd5gag MER682

THIMAGACA GAATGTCAGT CTTACAGTATA CATAGGTATA CATAGGTATA CATAGGTATA CATAGGTATA CATAGGTATA CATAGGTATA CATAGGTATA CATAGGTATA CATAGATAG CCGACGGCC CCGACGGCCC CCGACGGCCC ATTACATTAGA ATTACATTTAGA ATTACATAGA GTTACCTATA GTTACCTATA TTCACCTAATA TTCACCTAATA GTTACATTTAGA ATTACATTTAGA ATTACATATTAGA ATTACATATAGA ATTACATACATAGA ATTACATATAGA ATTACATACATAGA ATTACATACATAGA ATTACATACATACATAGA ATTACATACATACATAGA ATTACATACATACATAGA ATTACATACATACATACATAGA ATTACATACATACATACATAGA ATTACATACATACATACATACATACATACATACATACA	KCA GENCANATAN KCA GENCANATAN KUT TECCECCETI KCA AAGENCEAAA KUT GENCEGAAA KUT GENCEGAAA KUT GENCEGAAC KUT TECCAAC KUT TECC	CHGCHGCAC GTCCTCTGG TCCTGTCGT HGGACAGGG GGCCAGGAGG TCTATTACC AGATCAATGG ACCACCCCC AGGAAACCTA TCGTTTCGAT TCGTTTCGAT TCGTTTCGAT TCGTTTCGAT TCGTTTCGAT TCGTTTCGAT TCGTTTCGAT TTGGCCAGGA GCGATCGTCC GTCCCGGGAG GTCCCGGGAG GTCCCGGGAG GTCCCGGGAG GTCCCGGGAG GTCCCGGGAG GTCCCGGGAG GTCCCGGGAG GTCCCGGGAG GTCCCGGGAG GTCCCGGGAG GTCCCGGGAG GTCCCGGGAG GTCCCGGGAG GTCCCGGGAG GTCCCGGGAG GTCCCGGGAG GTCCCGCCC GTCCCGGGAG GTCCCGGGAG GTCCCGGGAG GTCCCGGGAG GTCCCGGGAG GTCCCGTC GTCCCGGGAG GTCCCGGGAG GTCCCGGGAG GTCCCGGGAG GTCCCGGGAG GTCCCTCCC GTCCCGGGAG GTCCCGGGAG GTCCCGGGAG GCCATTACAC GCCATTACAC GCCATTACAC	TCCTTGCCG GGCACCGGA SH SH TCCATTGCCA AMANACCA TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT	CCTTCCAGGT TATCTTGATG AAAGAATGAG AAAGAATGAG AAAGAATGAG AAAGAATGAG AAAGAATGAG AAAAAAATAATAA CAACAACAAG AACAACAAGAG AAAATAATGAT ATTAATTAATAATAATAATAATAATAATAATAATAAT	CTITATIATIOC CAACATTATIOC AACAACATTATIOC CACCCCTTITATIOC GAGGGANGA CAACATTACCCO TITATIACCCO TITATIACCCO GAGGGCANATA TITAGACCTITA CACCACACA GAGGANATA AACATACATA TITAGACCTATA TITAGACCTAC TATATATATA TITAGACCTAC TATATATATATA TITAGACCTAC TATATATATATATA TITAGACCTAC TATATATATATATATA TITAGACCTAC TATATATATATATATATATATATATATATATAT	AGCTTCCTCC TCGAGGGGGG ACTTCCGCGA ATCTCCCGAT TAGGGGGTTA TAGGGGGTTA TAGGGGGTTA TAGGGGGTTA TAGGGGGTTA TAGGGGGTTA TAGGGGGTTA TAGGCGAT TAGGCGAT TAGGCGAT TAGCCGAT TAGCCGAT TAGGCGAT TAGGGGTTA TAGGCGTTA TAGGGAT TAGGGAT TAGGGAT TAGGAT TAGGGAT TAGGAT TAGGGTA TAGGAT TAGGGTA TAGGTA TAGGGTA TAGGGTA TAGGTA TAGGGTA TAGGTA T	TOGCTOCTAL ANCIACOCTOT TICTOGCAGA GOGTFTCAAG CCCCAAGTTC CCTCACAGTTC CCTCACAGTT CCATCACAGTT TACATTACAGT TACATTACAT	CTTTCTCCAC GAAGAGGGG GAAGATACCT CTTCTATOGA AGAGTCCCCC TCTCAGGGG GGCCGGCAAC CCGGGCCGTTG ACTTAGATCGTA TATGATCGTA TTGATGGTTGA TCCTTACCCGA TCCTTACCCGA TCCTTACCCGA TCCTTACCCGA TCCTTACCCGA TCCTTACCCGA TCCTTACCCGA TCCTTACCGGA TCCTTACCGGA TCCTTACCGGA TCCTTACCGGA TCCTTACCGGA TTGATGGTTGAT TTTATGATGTTGAT TTTTATGATGTTGAT TTTATGATGTTGAT TTTATGATGAT TTTATGATGTTGAT TTTATGATGTTGAT TTTATGATGAT TTTATGATGTTATGAT TTTATGATGAT TTTATGATGTTATGAT TTTATGATGAT TTTATGAT TTTATGATGAT TTTATGATGAT TTTATGAT	ANTICTAAATG TEAACCCCTTAA AGTTGGGG A TGGGGTAGT : ACCCCATGAG GGAATGAGA TGCGCCTTGAG TGCGCCTTGAG TGCCACCTTT TGCGTGGGA TGCCACCTTT TGAATGAGA TGCCACCTTT TGCATGAT TGCATGAT TGCATGAT TTTTGATTCA TGCAAGTT TGCAAGTT TGCAAGTT TGCAAGTT TGCAAGTT TTTTGGCTAAA GGTAAACCCA GGAACTTCAA GGTAAAACCCA GGAACTTCAAA TTTTGGCTAAA TTTTGGCTAAA TTTTGGCTAAA
ATATCHEZAN TATAGACCTE B	ATATCTIZIA CAGITCAAG TATAGACCT GTCAAGTITC Bgil	-		TTCACGAMA		CTAMCAAIT CATTIGITAA		CCCAGAATAT	TL, JANCTITA ACCTIGNAAT
GAAATGGA CTTTACCT	GAAATGGAGA TCTTACTGAA CTTTACCTCT AGAATGACTT	CCCTCTCCCA	ATACANACGC	TGFTGGATTF	ATCCCTAACC	TATCACCTTA ATAGTCGAAT	TCCAAAATCT AGGTTTTAGA	CACCOUTAAAA	CTCCCAAAAG

Figure 15T

PMRKAd5gag MER682

32101	TAACATTGTC	AGTCAAGTTT TCAGTTCAAA	ACTTAAACCS TGAATTINGC	ACACAMANCT	AAACCTGTAA	CACTAACCAT	TACACTAAAC	GGTACACAGG	AMCAGGAGA	CACAACTC!.A GTGTTRIAG: 7'
32201	AGTOCATACT TCACGTATGA	CTATOTCATT	TTCATCCCAC ANGTACCCTG	Transporter Arcadactes	ACAM TACAT TOTTCATITA	TATTACTETAT	THYGCEACAT NACCEPTETA	CCTCTTACAC	TTTTTCATAC	ATTGCCCAN: TAACGCCTTP:
32301	AATAAAGAAT	CGITTIGAGETE	ATCHTTCAAC	CACAMATANA	THEANTHEEA	GAMATTECA	ACACTATATA TENTA	CATTCAGTAG	TATAGCCCCA ATATCGGGGT	CCACCACATA GGTGGTGTA1
32401	CENATATORE	ATCACCGTAC TAGTGGCATO	CTTAATCAAA	CTCACAGAAC	CCTAGTATTC	AACCTACCAC	CTCCCTCCCA	ACACACAGAG TGTGTGTCTC	TACACAGTCC	TTTK: TYCC: Y:
32501	GCTGGCCTTA	ANANGCATCA	TATCATCCC	AACAGACATA	TTCTTMGGTG		CACAGITTICE	TGTCGAGGCA	AACGCTCATC TTGCCAGTAG	AGTONTATT TCACTATA
32601	ATAAACTCCC TATTTGAGGG	COCCACCIC	ACTTAAGTTC TGAATTCAAG	ATCTCCCTCT TACACCGACA	כבאיא־זמכיזק מבזעמאכיאכ		TOCTGTCCAA	CTTCCCGGTTG	CTTAACGGGC	CCCCTTCCT
32701	AAGTECAGGE	CTACATOGGG GATGTACCCC	GTAGAGTCAT	AATCCTCCAT	CAGGATACAG GTCCTATCCC		CHITHSTACT GCAGCAGCUC	GCGAATAAAC	TGCTGCCGCC ACGACGCCGG	OCCUPATION OF CONTRACT CONTRAC
32801	Patt CCTGCAGGAA GGACGTCCTT	TACAACATGG	CAGNOGICTC	CTCAGCGATG	ATTCGCACCG TAAGCGTCAC	CCCGCAACAT	AAGGCGCCTT TTCCGCCGCAA	GTCCTCCGGG	CACAGCAGGG GTGTCGTCGC	CACCCTOAT C
32901	TCACTTAAAT AGTGAATTTA	CAGCACAGTA	ACTGCAGCAC TCACCTCGTG	AGCACCACAA	TATTCTTCA ATACAGTT	AATCCCACAG	TGCAAGGCGC	TGTATCCAAA ACATAGGTTT	GCTCATGGCG	GOGACCACAG
33001	AACCCACGTG	GCCATCATAC CGGTAGTATG Kprf	CACAAGCGCA	GCTAGATTAA CCATCTAATT	GTMRCRACCC CACCGCTCGG	CTCATAAACA GAGTATTTGT	CCCTCGACAT	ANACATTACC TITGTAATGG	TCTTTTGGCA AGAAAACCGT	TCTTCTAATT ACAACATTAA FSII
33101	CACCACCTCC GTGGTGGAGG Pbil	00		TABACCTCTG ATTABACATG	GCGCCATCCA	CCACCATCCT	NAACCAGCTO	GCCAAAACCT COGTTTTGGA ExoftV	000000000	TATACACTUS: ATATUSTGAC:
33201	ACCETTORCE	CACTGGAACA		ATGACAGITIG AGAGCCCAGG TACTGTCACC TCTCGGGTCC	ACTOSTANCO TOACCATTOO	ATGGATCATC TACCTAGTAG	ATCCTCGTCA TACGAGCAST	TGATATCAAT	GTTGGCACAA	CACAGGCACA GTGTCGTGT PSI
33301	CCTCCATACA	CTTCCTCAGG	ATTACAAGCT TAATGTTCGA		CCTCCCCCT TAGAACCATA GGARGCCCA ATCTFGTAT	TCCCAGGAA	CAACCCATTC	CTGAATCAGE	GTAAATCCCA CATTTAGGGT	CACTGCAGGG
33401	ANGACCTCCC TTCTCGAGCC	ACCITACTCA TOCATTCAGT	CGTTGTGCAT	TGTCANAGTG ACAGT TTCAC	TTACATTCGG AATGTAAGCC	CACAGCAGCAG CCTCGTCGCC	ATGATCCTCC TACTAGRIAGG	AGTATOGTAG TCATACCATC	CCCCCCAAG	TOTOTOAN ACAGAGITT
33501	GGAGGTAGAC CCTCCATCTG	GATCCCTACT CTAGGGATGA	GTACGGAGTG	CCCCCCATACA	ACCCAGATCG TOGCTCTAGC	TGTTKGGWGT ACAACCAACA	AGTGTCATTC TCACAGTACG	CANATOGNAC	OCCOCACOTA COCCTOCAT	GTCATAITTH CAGTATAAAK!

- Annual

33601	CTCAACCAAA	ACCAGGITGE	GOCGTRACAA	ACM:ATCTRIC (פוניונכנימב	TryCCCC: ##A (GATCCCTCTC 1			CACTCTCTVA
	GACTICOTITY		CCGCACTIVITY	TCTCTAGACG	נאנזאנזוננאנז	ACCOCCAAT (CTARCCIAGAC 1	ACATCATCAA (CATCATATAG	CTCAGAGACT"
33701	AAGCATCCAG	GCCCCCTO	CONCOUNT	CTATGTANAC '	TCCTTCATGG	, טניניניניניניניני	_		-	CACCCAGGC!
	TEGTAGGIC	COCCOCCCAC	CGAAGCCCAA	GATACATTEG	AGGAAGTACG	COULCACOOG	ACTATTOTAG	STOCTOCCOT (CTTATTCGGT	Green com
33801	ACCTACACAT	restrence	AGTCACACAC	CCCARRANCCC	GGAAGAGCTTG	CAACAACCAT	GRAPHTER .	TTATTCCAAA	AGATTATCCA	AVACCT1 VAA
	TGGATGTGTA				CCTTCTCGAC	CTICTINGTA	CUNTANAAA	AATAAGGTTT	TCTANTAGGT	TTTCCAGTTT
	Bgff									
13901	ATGAAGATCT	ATTAAGTGAA	COCOCTOCCC	Tecantrace	TYGICAAACT	CTACAGCCAA	NGNACAGNTA	ATCCCAPTTC	TANGATETTO	CACMIDGCT
	TACTTCTAGA	TAATTICACTT	GCCCGAGGGG	ACCCCACCC	ACCAGITICA	GATGTCGGTT	TCTTGTCTAT	TACCGTANAC		GTGTTINCCGI
34001	TCCAAAAGGC	AAACGCCCCT	CACGTCCAAG	TEGACGTAAA	GGCTANACCC	TTCAGGGTGA	ATCTCCTCTA	TAMACATTCC	ACCACCTTCA	ACCATGCCCA
	AGGITTICCO	TTTGCCGGGA	OTGCAGGTTC	ACCTGCATTT	CCGATTTCCC	NAGTCCCACT	TACACCACAT	ATTTOTAGG	TCGTCGAACT	TOGTACOOCT
14101	AATAATTCTC	ATCTCGCCAC	CTTCTCANTA	TATCTCTAAG	CAANTCCCGA	ATATTAAGTC	CGGCCATHGT	AAAAATCTGC	TCCAGAGGGG	CCTCCACCTT
	TTATTAAGAG	-	GAAGAGTTAT	ATAGAGATTC	GTTTAGGGCT	TATAATTCAG	CCCCCTAACA	THITTAGACG	AGGICICOCO	GCAGCTGCAA
34201	CAGCCTCAAO	CAGCGAATCA	TGATTGCAAA	AATTCAGGTT	CCTCACAGAC	CTCTATANGA	TTCANAGCG	GAACATTAAC	AAAAATACCO	CGATCCCGTA
	GICOGAGITIC		ACTAACGITT	TTAAGTCCAA	GGAGTGTCTO	GACATATTCT	AAGITITICGC	CITICIAATIG	TTTTTATOGC	GCTAGGGCA1
34301	CONCECTION	CAGGGCCAGC	TGAACATAAT	COTTACAGGTC	TGCACGGACC	AGCGCGGCCA	כיווכככככככ	ACCAACCATG	ACANAGGAG	CCACACTOAT
 - -	CCAGGGAAGC	Greceoenco	ACTIGITATIFA	GCACGTCCAG	ACGTGCCTGG TCGCGCCGGT	TCCCCCCGGT	GAAGGGGCGG	1cc11CC1AC	Territerio	GGTGTCACTA
					Hindl	191				
34401	TATEACAGGC	_	ATACTCOGAG 'CTATOCTAAC	CAGCGTAGCC	CCCATGTANG CTTGTTGCAT	CTTGTTGCAT	GGCCGCCGAT	ATAMATGCA	AGGRECTECT	CAAAAAATC
	ATACTGTGC	TATGAGCCTC	GATACGATTG	GTCGCATCGG	COCTACATTC	GAACAACGEA	CCCGCCGCTA	TATITIACGE	TCCACGACGA	GPPFFFFACE.
34501	OCCAMBEE	COCCCAAAAA	ACIAAAGCACA	TCGTAGTCAT	CCTCATGCAG	NTANARGENG	GTAAGCTCCG	GNACCACCAC	AGAMANAGAC	ACCAPITITIC
	CCGTTTCOGA	OCCOUNTIFF.	remiconst	AGCATCAGTA	CCACTACGTC	TATTTCCGTC	CATTICGAGGC	CITIGGICONG	TCTTTTTCTG	TCCTANAAAG
34601	TCTCAMCAT	OPETICOCOCOF	TTCTGCATAA	ACACAMATA	ANATAACANA	MAACATTTA	AACATTAGAA	GCCTGTCTTA	CAACAGGAAA	AACAACCC7'T
	AGACTITICTA	CAGACOCCCA	AAGACGTATT	TCTCTTTAT	TITATICITY	TITIGINAL	TIGINATOTI	COCHCACAAAT	OFIGICCITY	TETTOCGAA
34701	ATANGCATAA	GACCGCACTAC	GCCCATGCCG	CCCTGACCGT	MANAMARCTG	GTCACCGTGA	TTAMAMOCA	CCACCCACAG	CHECHEGRATE	ATGRECCOOMS
	TATTCGTATT	CTGCCTGATO	CCCCTACGCC	CCCACTCCCA	TETETTEAC	CASTGGCACT	ATTITITEGE	GGTGGCTGTC	GAGGAGCCAG	TACAGACCTC
34801	TCATAATOTA	AGACTCOOTA	AACACATCAG	GPTGATTCAC	ATCOSTCAGT	GCTAANNGC	GACCGANATA	CCCCCCCCCCCA	ATACATACCC	GCAGACAG
	AGTATTACAT	TCTGAGCCAT	TIGICITAGIC	CAACTAACTG	TAGCCAGTCA	CCATTITION	CTGGCTTTAT	COCOCCCCT	TATGTATOCG	CONCOCANO
34901	AGACAACATT	ACAGCCCCCA	TAGGAGGTAT	AACAMATTA	ATAGGAGAGA	ANANCACATA	AACACCTICINA	ANACCCHICCT	GCCTARGCAN	AATAGCACCC
	TCTGTTGTAA			TICITITANT	TATECTETET	THINGTAL	TTGTCCACTT	TTTCCCACCA	COCATCCUT	TTATCGTGGG
35001	TCCCCCTCCA	GANCAACATA	CAGCGCTTCC	ACAGCGGCAG	CCATAACAGT	CAGCETTARCE	ACTANAAAG	AAAACCTATT	MANAMACAC	CACTCGACN:
	ACCCCCACCT	CITCHIGHTOTAL	GTCGCGAAGG	reredecent	GGTATTOTCA	CHUCCAATCC	TCATTFTFTC	TEFFICEATAA	THITTELE	GROAGCTGINS
35101	COCACCAGCT	· CAATCAGTCA	CAGTGTANAA	AAGOGCCAAG	TGCAGAGCGA	CTATATATAG	GACTAAAAAA		GTTAAAGTCC	ACAAAAACA
	CCGTGGTCGA	GITTAGTCAGT	GICACAITIT	TTCCCGGTTC	ACGRETERE	CATATATATC	CICATIFIE	ACTOCATTOC	CAATTICAGG	16truner
35201	CCCAGAAAAC	COCACOCOAN	CCTACGCCCA	GANACCANAG	CCANNANCC	CACAACTTCC	TEMANTICOTE	ACTICCGTUT	TCCCACGITA	CONCACTICC
	GGCTCTTTTG	_	GGATGCGGGT	CTTRICCTTTC	GCTTT-TT-TGG	GTCTTGAAGG	AGTITAGCAG	TCAAGGCAAA	AGGGTGCAAT	GCAGTGAAGG

Figure 15V

PMRKArl5gag MER682

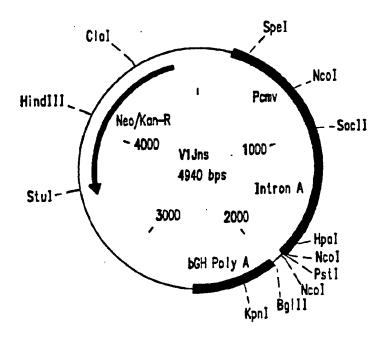
35301 C	CATTITANGA	AAACTACAAT	TCCCAACACA	TACAAGTTAC	TYCCYCCTAA AGGCGGGATT	ANCETACETE ACCEGECECE THEGATECIA: TEXACOGOGE		TTCCCACGCC (AAGGCTTCCGG (AAGGCTTCCCGG (AAGGCTTCCGG (AAGGCTTCCCGG (AAGGCTTCCCGG (AAGGCTTCCCGG (AAGGCTTCCCGG (AAGGCTTCCCGG (AAGGCTTCCCGG (AAGGCTTCCCG) (AAGGCTTCCCGG (AAGGCTTCCCG) (AAGGCTTCCCGG (AAGGCTTCCCG) (AAGGCTTCCCG) (AAGGCTTCCCGG (AAGGCTTCCCGG (AAGGCTTCCCG) (AAGGCTTCCCGG (AAGGCTTCCCG) (AAGGCTTCCCG) (AAGGCTTCCCG) (AAGGCTTCCCG) (AAGGCTTCCCG) (AAGGCTTCCCGG (AAGGCTTCCCG) (AAGGCTTCCCCG) (AAGGCTTCCCG) (AAGGCTTCCCCG) (AAGGCTTCCCCG) (AAGGCTTCCCCG) (AAGGCTTCCCCG) (AAGGCTTCCCCG) (AAGGCTTCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC	CCCCCCACG GCCCCCACG	TCACAAACTC AGTGTTTGAG
O	GTAMATICE		AGGGTTGTGT	ATGITCAATG		Ė				
•						D84	Fcofil			
35401 C	CACCCCCTCA	AATAGTATA	GCCTTCAATC	CANANTANGG	TATATTATTG	ATCATCTTAA TACTACAATT	•	GCATCTGCGA CCTAGACGCT	COCCAGGCTO	GATCGCCTT .
35501 0	CCCATTATOA	THEFTETEGE	TTCCGCGGC	ATCGGGATGC	CCGCGTTGCA	COCCATOCTO .	TCCAGGCAGG	TAGATGACGA	CCATCAGGGA	CACCTTCAAG
35601 0	CCACCAAA		CCTAAAAAGG	CCCCOTTCCT	GGCGTTTTTC					GCTCAAGTC7.
35701 0	CACCACCACA		GACTATAAAG		TTTCCCCCTG		CGROCGCTCT	CCTGTTCCGA	CCCTGCCGCT	TACCOGATAC ATGCCCTATY:
35801 0	CTGTCCOCCT	TICTCCCTTC	GCGAAGCGTG CCCTTCGCAC		ATARCTCACG TATCGAGTGC		CTCANTICOG	TOTAGGTCGT	TCGCTCCAAG AGCGAGGTTC	CTCCCCTCT.
35901 1	TUCACOAACC	CCCCGTFCAG	CCCGACCGCT	GCGCCTTATC	CCCATTCATA	CCTCTTGAGT	CCAACCCGGT	AAGACACGAC	TTATCOCCAC	TOCCACCAGY: ACCGTCGTC
36001 C	CACTOGTAAC	AGGATTAGCA TCCTAATCGT	GAGCGAGGTA	TCTAGCCGGT ACATCCGCCA	GETACAGAGT CGATCTCTCA	TCTTGAAGTG AGAACTTCAC	GTOCCCTAC	TACOGCTACA ATGCCGATOT	CTAGAAGGAC	AGTATTTGGF TCATAAACCA
36101	ATCTOCOCTC TAGACGCGAG	TOCTGAAGCC	AGITACCITIC	CCTTTTTCTC	THESTAGETE	TTGATCCGGC	ANACANACCA	CCCCTGGTAG	CCCTCCTTTT	TTTGTTTGC. AAACAAACGT
36201	AGCAGCAGAT PUCTUCOTOTA	TACGCGCAGA	AAAAAAGGAT	CTCAAGAAGA	TCCTTTGATC	AMMGATGC	CCAGACTIACG	TCAGTGGAAC AGTCACCTTG	GAMAACTCAC	GTTAAGGCAT
36301	TTTOOTCATO AAACCAGTAC	AGATTATCAA	AAAGGATCTT	CACCTAGATC	CTTTTAAATC	ANTCTANAGE	ATATATGAGT TATATACTCA	ANACTICORC	TGACAGTTAC ACTGTCAATG	CAATACTEAN
36401	TCAGTGAGGC AGTCACTCCG		GCGATCTGTC	TATTICGITIC	ATCCATAGTT TAGGTATCAA	GCCTGACTCC	CCGTCCTGTA	GATAACTACG	ATACGGGAGG TATGCCCTCC	GCTTACCATY CCAATAG
36501	TOCCCCAOT	OCTOCANTOA CGACGTTACT	TACCOCCAGA ATGCCGCTCT	CCCACGCTCA	CCGGCTCCAG	ATTTATCAGE TAAATAGTEE	AATAAACCAG TTATTTGGTC	CCAGCCGGAA	GCGCCGAGCG	CAGNACTOGT
36601	CCTCCACT	TATCCOCCTC ATAGGCGGAG	CATCCAGTCT	TAATTAACAA	GCCGGGAAAC	TAGACTEATICA	AGTTCGCCAG TCAAGCGGTC	TTAATAGTTT AATTATCAAA	GCGCAACGTT CGCGTTGCAA	GITGCCATTG
36701 0	CTACAGGCAT GATOTCCGTA	CCTCCTCTCA	CCCTCCTCGT	TIGGTATGCC	TTCATTCAGC AAGTAAGTCG	TCCGGTTCCC AGGCCAAGGG	AACCAACTIC TTGCTAGTTC	GCGAGTTACA CGCTCAATGT	TCATCCCCCA ACTAGGGGGT	TOTTGTCCAA ACAACACGTT
				, m						
36801	MANGEGET	AGCTCCTTCG TCGAGGAAGC		GICCTCCGAT COTTGICAGA CAGGAGGCTA GCAACAGTCT	ACTANGTING TCATTCANCC	CCCCACTCTT	ATCACTCATG TAGTGAGTAC	CANTACCOTC	CACTGCATAA	TICKCITACT AAGAGAATGA
36901	GICATGCCAT	CCGTAAGATG		CTTITCTOTO ACTOCITIAGE	ACTUMCCAA TGAGTTGGTT	CAGTAAGACT	GAATAGTGTA	GAATAGTGTA TOCGGGGACC	CTCAACGAGA	Traccompage

figure 15W

PMRKAd5gag MER682

CANCACCOGA TANTACCOCO CCACATAGO, GAACTITIANA AGRACTCATC, ATTORANAC GITCTFOGGO GCOADAACTE TCAAGGATCT TACKYCTYTT GITGTGCCCT ATTATGACGO GGTGTATGGT CITKAAATTT TKAGGARTAG TAACCTTTTG CAAGAAGGCC GGCTTTTGAG AGTTCCTAGA ATGGGGAGAA	GAGANCEAGT TOCANTATAR CARCIONIC ACTAMENTA TOTHINNANT CHITTACTT CACAMEGET TOTAGETISAG CAANAACAG ARGCANAAT CHITTAGECA AGETACATTO GETREATHERE TYCKHETHE GAANAHAAA GETREATHERE TYCKHETHE	SCATITIA TCAGRITTAT TCHCTCATGA GENERAL GOTAAAT ACACCCATA ACAGAGTACT	GOGGATACAT ATTTGAATGT ATTTAGAAA ATAAACAAAT AGGGGTTTCG GCGTGTAAG GGGCTTTTCA COGGGGACTG GTCTAAGAAA CCATTATTAA GGCCTATGTA TAAACTTACA TAAATCTTTT TATTTGTTTA TCCCCAAGGG GCGTGTAAAG GGGCTTTTCA CGGGGACTG CAGATTCTTT GGTAAATA	r (SEQ ID NO: 27)
CANCANGREC COC	CACCAGGITT TOTAL	CANTAITATT GAN	CCCGAAAAGT GCC	E-DATH Bamfil GGA TUCGAATTUT TAN CCT AGGCTTAAGA ATT
NEATE ATTOCAMANC	איזידא בידידר באיצא איזידא באאאזנאאא	NEACT CTRECTEPTE	וודכה היקאראדאה אתמכ פכמומדאאא	BA MANG TECTANGER
INCTITIONA AGRICO.	KYTTYL ASTINATYS KXYYTIGACY AGAAKI	ANTGITIGAA TACTICI TTACAACTI ATGAGI	TAAACAAAT AGGGGG	ACGARACCC TTTCG: TGCTCCRGG AAAGC)
CCCC CCACATAGGA COCCCC CCTCTATCGT C	OTANG CCACTCGTGC /	ATNAC COCCACACOS /	AATGT ATTTAGAAA / TTACA TAAATCTTTT 1	EGRITI CATGACATTA ACCTATAAAA ATAGGGGTAT CACGAGGCC TTTCGTCTTC AAGAATTGA TTCGAATTCT TAAT (SEQ ID NO: 27) GTACTGTAAT TGJATATTT TATCCGCATA GTGCTCCGGG AAAGCAGAAG TTCTTAACCT AGGCTTAAGA ATTA (SEQ ID NO: 28)
CAACACGGGA TAATA GTTGTGCCCT ATTAT	GAGATECAGT TEGAT CTCTAGGTEA AGETA	CCCCOTTITI TCCT	GCGGATACAT ATTTG CGCCTATGTA TAAAC	CATGACATTA ACCTA GTACTGTAAT TGGAT
			37301	37401

Figure 15X



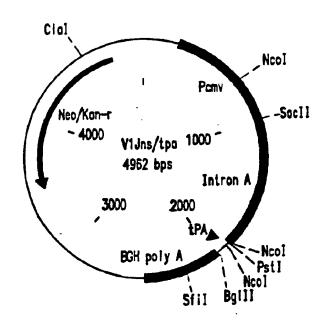


FIGURE 16

GCAGTGGCCCCTGACTGAGGAGAAGATCAAGGCCCCTGCTGGAAATCTGCACTGAGATGGAGAAGGAGGGGCAAAATCTCCA
sGInTrpProLeuThrG1uG1uLysI1eLysA1oLeuVo1G1uI1eCysThrG1uMetG1uLysG1uG1yLysI1eSerL
30 40 50

AGATIGGCCCCGAGAACCCCTACAACACCCCTGTGTTTGCCATCAAGAAGAAGGACTCCACCAAGTGGAGGAAGCTGGTG
yslieGlyProGluAsnProTyrAsnThrProVolPheAiolieLysLysAspSerThrLysTrpArgLysLeuVol
60 70

GACTICAGGGGGCTGAACAAGAGGACCCAGGACTTCTGGGAGGTGCAGCTGGGCATCCCCCACCCCGCTGGCCTGAAGAA AspPheArgGIuLeuAsnLysArgThrGInAspPheTrpGIuVoIGInLeuGIyIIeProHisProAloGIyLeuLysLy 80 90 100

GAAGAAGTCTGTGACTGTGCCGGCTGTGCCCGATGCCTACTTCTCTGTGCCCCTGGATGAGGACTTCAGGAAGTACACTG slyslysSerVolThrVolLeu<u>Alo</u>VolGlyAspAloTyrPheSerVolProLeuAspGluAspPheArgLysTyrThrA 110 120 130

CCTTCACCATCCCCTCCATCAACAATGAGACCCCTGGCATCAGGTACCAGTACAATGTGCTGCCCCAGGGCTGGAAGGGC IoPheTnrlleProSerlleAsnAsnGluThrProGlylleArgTyrGlnTyrAsnVolLeuProGlnGlyTrpLysGly 140 150

TCCCCTGCCATCTCCAGTCCTCCATGACCAAGATCCTGGAGCCCTTCAGGAAGCAGAACCCTGACATTGTGATCTACCA SerProAioliePheGinSerSerMetThrLyslieLeuGiuProPheArgLysGinAsnProAsplieVoilleTyrGi 160 170 180

GTACATGGCTGCCCTGTATGTGGGCTCTGACCTGGAGATTGGGCAGCACAGGACCAAGATTGAGGAGCTGAGGCAGCACCC
nTyrMetAloAloLeuTyrVo1G1ySerAspLeuG1uIleG1yG1nHisArgThrLysIleG1uG1uLeuArgG1nHisL
190 200 210

TCCTGAGGTGGGGCCTGACCACCCCTGACAAGAAGCACCAGAAGGAGCCCCCCCTTCCTGTGGATGGGCTATGAGCTGCAC euleuArgTrpGTyLeuThrThrProAsplysLysHisGInLysGIuProProPheleuTrpMetGTyTyrGTuLeuHis 220 230

CCCGACAACTGGACTGTGCACCCCATTGTGCTGCCTGAGAAGGACTCCTGGACTGTGAATGACATCCAGAAGCTGGTGGG ProAspLysTrpThrVoiGinProIieVoiLeuProGiuLysAspSerTrpThrVoiAsnAspIieGinLysLeuVoiGi 240 250 260

CAAGCTGAACTGGGCCTCCCAAATCTACCCTGGCATCAAGGTGAGGCAGCTGTGCAAGCTGCTGAGGGGCACCAAGGCCC yLysLeuAsnTrpAloSerGinlieTyrProGiylleLysVolArgGinLeuCysLysLeuLeuArgGiyThrLysAloL 270 280 290

FIGURE 17A

GGGGTGTACTATGACCCCTCCAAGGACCTGATTGCTGAGATCCAGAAGCAGGGCCAGGGCCAGTGGACCTACCAAATCTA GiyVoiTyrTyrAspProSerLysAspLeulieAloGiulieGinLysGInGlyGInGlyGInTrpThrTyrGInlieTy 320 340

CCAGGAGCCCTTCAAGAACCTGAAGACTGGCAAGTATGCCAGGATGAGGGGGGCCCACACCAATGATGTGAAGCAGCTGA rGinGluProPheLysAsnleuLysThrGiyLysTyrAlaArgMelArgGiyAloHisThrAsnAspVoiLysGinLeuT 350 360 370

CTCAGGCTGTGCAGAAGATCACCACTGAGTCCATTGTGATCTGGGGCAAGACCCCCAAGTTCAAGCTGCCCATCCAGAAG hrGluAloVolGinLyslleThrThrGluSerlleVollleTrpGlyLysThrProLysPheLysLeuProlleGinLys 380 390

GGTGAAGCTGTGGTACCAGCTGGAGAAGCAGCCCATTGTGGGGGGCTGAGACCTTCTATGTGGCTGGGGCTGCCAACAGGG uVollysleuTrpTyrGinleuGiuLysGiuProlleVolGlyAloGluThrPheTyrVolAloGlyAloAloAsnArgG 430 440 450

AAGACTGCCCTCCAGGCCATCTACCTGGCCCTCCAGGACTCTGGCCTGGAGGTGAACATTGTGACTGCCTCCCAGTATGC
LysThrAioleuGinAloileTyrLeuAioleuGinAspSerGiyLeuGiuVolAsnIieVolThrAioSerGinTyrAi
480
490
500

CCTGGGCATCATCCAGGCCCAGCCTGATCAGTCTGAGTCTGAGCTGGTGAACCAGATCATTGAGCAGCTGATCAAGAAGG DLeuGlyItelleGinAloGinProAspGinSerGluSerGluLeuVolAsnGinItelleGluGinLeuItelysLysG 510 520 530

AGAAGGTGTACCTGGCCTGCCCGCCCACAAGGCCATTGGGGGCAATGAGCAGGTGGACAAGCTGGTGTCTGCTGGC
IULysVoITyrLeuAIoTrpVoIProAIoHisLysGIyIIeGIyGIyAsnGIuGInVoIAspLysLeuVoISerAIoGIy
540
550

ATCAGGAACGTGCTGTTCCTGGATGCCATTGACAAGGCCCAGGATGAGCATGAGAAGTACCACTCCAACTGGAGGGCTAT

11eArgLysVolleuPheleuAspGiyI1eAspLysAloGInAspGluHisGluLysTyrHisSerAsnTrpArgAloMe

560 570 580

FIGURE 17B

GGCCTCTGACTTCAACCTGCCCCCTGTGGTGGCTAAGGAGATTGTGCCCTCTGTGACAAGTGCCAGCTGAAGCCGGAGG tAloSerAspPheAsnLeuProProVolVolAloLysGiulleVolAloSerCysAspLysCysGinLeuLysGlyGluA 590 600 610

GCTGTGCATGTGGCCTCCGGCTACATTGAGGCTGAGGTGATCCCTGCTGAGACAGGCCAGGAGACTGCCTACTTCCTGCT AlovotHisVotAloSerGlyTyrIleGluAloGluVoilleProAloGluThrGlyGlnGluThrAloTyrPheLeuLe 640 650 660

GAAGCTGGCTGGCAGGTGGCCTGTGAAGACCATCCACACTGCCAATGGCTCCAACTTCACTGGGGCCACAGTGAGGGGTG uLysLeuAloGlyArgTrpProVolLysThrlleHisThrAloAsnGlySerAsnPheThrGlyAloThrVolArgAloA 570 680 690

CCTGCTGGTGGGCTGGCATCAAGCAGGAGTTTGGCATCCCCTACAACCCCCAGTCCCAGGGGGTGGTGGCCTCCATGAAC IoCysTrpTrpAloGlylleLysGInGluPheGlylleProTyrAsnProGInSerGinGlyVolVolAloSerMetAsn 700 710

AAGGAGCTGAAGAAGATCATTGCGCAGGTGAGGGACCAGGCTGAGCACCTGAAGACAGCTGTGCAGATGGCTGTTCAT LysGluLeuLysLyslielleGlyGinVolArgAspGlnAloGluHisLeuLysThrAloVolGlnMetAloVolPhell 720 730 740

CCACAACTTCAAGAGGAAGGGGGGCATCGGGGGGCTACTCCGCTGGCGAGAGGATTGTGGACATCATTGCCACAGACATCC
eHisAsnPheLysArgLysGlyGlyIleGlyGlyTyrSerAloGlyGluArglleVolAspIleIleAloThrAspIleG
750
760
770

AGACCAAGGAGCTCCAGAAGCAGATCACCAAGATCCAGAACTTCAGGGTGTACTACAGGGACTCCAGGAACCCCCTGTGG
InThrLysGIuLeuGInLysGIn!ieThrLysIieGInAsnPheArgVoITyrTyrArgAspSerArgAsnProLeuTrp
780 790

AAGGGCCCTGCCAAGCTGCTGTGGAAGGCCGACGGGGCTGTGGTGATCCAGGACAACTCTGACATCAACCTGGTGCCCAG LysGTyProAtoLysLeuLeuTrpLysGTyGTuGTyAtoVotVotTteGtnAspAsnSerAspTteLysVotVotProAr 800 810 820

AAAGCCCCGGCCAGATC" (SEQ ID NO: 3)
Xx Bg/ll (SEQ ID NO: 4)

FIGURE 17C

CCACCCACATCTCCCCCCATCTCCCCATTCACACTGTCCTGTCAAGCTGAAGCTGCCATGGC (within SEO 10 NO: 7)
RoSerCivileSerAloProlleSerProlleCluThrValProVolLysLeuLysProClyMetAspGly (within SEQ 10 NO: 8)
-1 2 70

FIGURE 18

WT	- ATG GGT GGC AAG TGG TCA AAA CGT AGT GTG CCT GGA TGG TCT -42
OPT	M G G K W S K R S V P G W S -14
WT OPT	- ACT GTA AGG GAA AGA ATG AGA CGA GCT GAG CCA GCA GCA GAT -84
	T V R E R M R R A E P A A D -28
WT OPT	- AGG GTG AGA CGA ACT GAG CCA GCA GCA GTA GGG GTG GGA GCA -126
WT	- GTA TCT CGA GAC CTG GAA AAA CAT GGA GCA ATC ACA AGT AGC -168
OPT	II II
WT	- AAT ACA GCA GCT ACC AAT GCT GAT TGT GCC TGG CTA GAA GCA -210
OPT	- AAC ACC GCC GCC ACC AAC GCC GAC TGC GCC TGG CTG GAG GCC N T A A T N A D C A W L E A -70
WT	- CAA GAG GAT GAG GAA GTG GGT TTT CCA GTC AGA CCT CAG GTA -252
OPT	- CAG GAG GAC GAG GTG GGC TTC CCC GTG AGG CCC CAG GTG Q E D E E V G F P V R P Q V -84
WT	- CCT TTA AGA CCA ATG ACT TAC AAG GGA GCT GTA GAT CTT AGC -294
OPT	- CCC CTG AGG CCC ATG ACC TAC AAG GGC GCC GTG GAC CTG TCC P L R P M T Y K G A V D L S -98
WT_	- CAC TIT TTA AAA GAA AAG GGG GGA CTG GAA GGG CTA ATT CAC -336
OPT	- CAC TTC CTG AAG GAG AAG GGC GGC CTG GAG GGC CTG ATC CAC H F L K E K G G L E G L I H -112
WT	- TCA CAG AAA AGA CAA GAT ATC CTT GAT CTG TGG GTC TAC CAC -378
OPT	- TCC CAG AAG AGG CAG GAC ATC CTG GAC CTG TGG GTG TAC CAC S Q K R Q D I L D L W V Y H -126
WT	- ACA CAA GGC TAC TTC CCT GAT TGG CAG AAC TAC ACA CCA GGG -420
OPT	- ACC CAG GGC TAC TTC CCC GAC TGG CAG AAC TAC ACC CCC GGC T Q G Y F P D W Q N Y T P G -140

FIGURE 19A

WT	- CCA GGA ATC AGA TTT CCA TTG ACC TTT GGA TGG TGC TTC AAG -462	
OPT	- CCC GGC ÁTC ÁGG TTC CCC CTG ÁCC TTC GGC TGG TGC TTC AAG P G I R F P L T F G W C F K -154	
WT	- CTA GTA CCA GTT GAG CCA GAA AAG GTA GAA GAG GCC AAT GAA -504	
OPT	- CTG GTG CCC GTG GAG CCC GAG AAG GTG GAG GAG GCC AAC GAG L V P V E P E K V E E A N E -168	
WT	- GGA GAG AAC AAC TGC TTG TTA CAC CCT ATG AGC CAG CAT GGG -546	
OPT	- GGC GAG AAC AAC TGC CTG CTG CAC CCC ATG TCC CAG CAC GGC G E N N C L L H P M S Q H G -182	ı
WT	- ATA GAG GAC CCG GAG AAG GAA GTG TTA GAG TGG AGG TTT GAC -588	ļ
OPT	- ATC GAG GAC CCC GAG AAG GAG GTG CTG GAG TGG AGG TTC GAC 1 E D P E K E V L E W R F D -196	•
WT .	- AGC AAG CTA GCA TIT CAT CAC GTG GCC CGA GAG CTG CAT CCG -630)
OPT	TCC AAG CTG GCC TTC CAC CAC GTG GCC AGG GAG CTG CAC CCC S K L A F H H V A R E L H P -210)
WT	- GAG TAC TAC AAG GAC TGC TGA (SEQ ID NO:30) -65:	l
OPT	- GAG TAC TAC AAG GAC TGC TAA (contained within SEQ ID NO: 9) E Y Y K D C (SEQ ID NO: 10) -21	5

FIGURE 19B

VIJns/nef

CATGGGTCTTTTC<u>IGCAG</u>TCACCGTCCTTGAG<u>ATCI</u>GCCACC ATG GGC GGC AAG TGG TCC ANG AGG TCC GTG CCC . .

Srf1 Bg111
. . . CAC CCC GAG TAC TAC AAG GAC TGC TAA AGCCCGAGCAGAICIGCTGCCTTCTAGTTGCCAGC (SEQ 1D NO: 38)
H P E Y Y K D C * (contained within SEQ 1D NO: 10:

V1Jns/nef(G2A.LLAA)

Psti Catrbasticttttgigagicaccstgaga<u>ict</u>tscacc atg gcc ggc aag tgg tgc gtg ccc . M A G K W S K R S V P

Srff BgIII . . . CAC CCC GAG TAC TAC AGG GAC TGC TAA AGCCCGGGGAGAICIGCTGCCTTCTAGTTGCCAGC (SEQ 1D NO: 39) H P E Y Y K D C * (contained within SEQ 1D NO:14)

ViJns/tpanef & ViJns/tpanef(LLAA)

CTG CTG CTG TGT GGA GCA GTC TTC GTT TCG CCC AGC GAG AIC ICC TCC AAG AGG TCC GTG CCC ...

. . . . CAC CCC GAG TAC TAC AAG GAC TGC TAA AGCCCGGGGGGGCTGCTGCTGGCCAGC (SEQ ID NO: 40)

H P E Y Y K D C * (contained withon seq id no: 16)

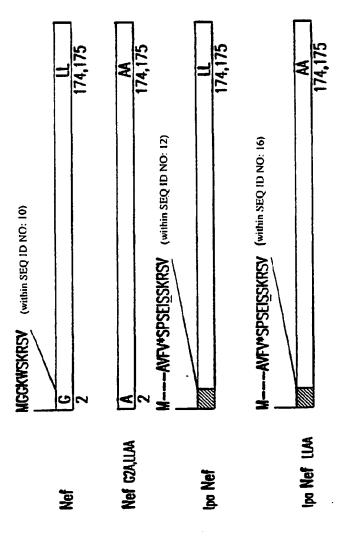


FIGURE 21

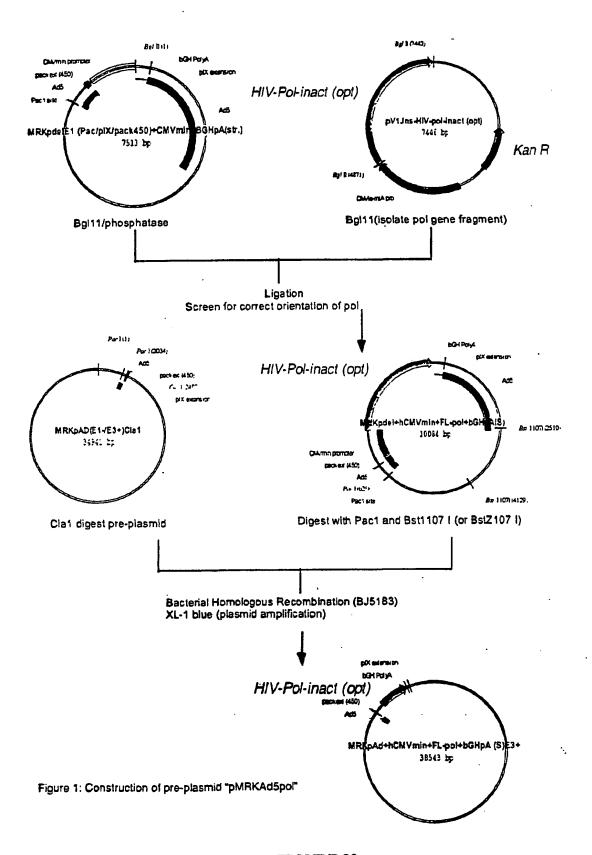


FIGURE 22

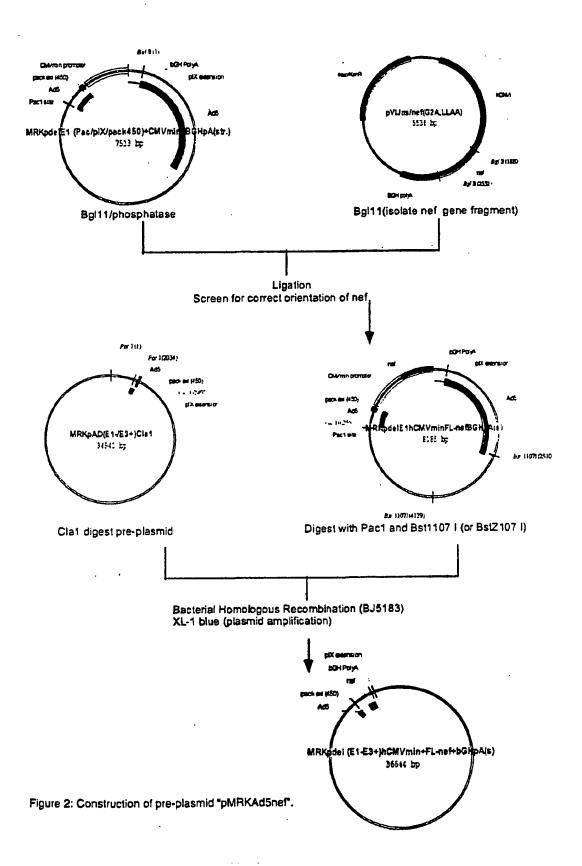


FIGURE 23

Comparison of Clade B vs. Clade C Anti-gag T Cell Responses in Clade B HIV-Infected Subjects

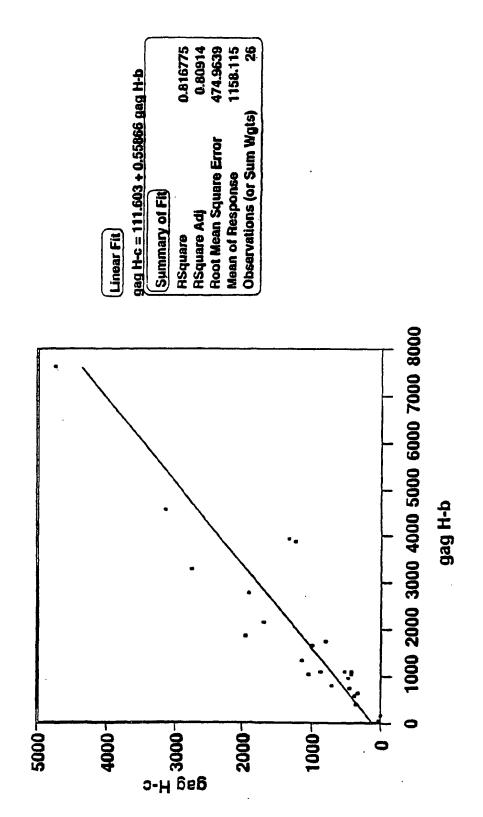
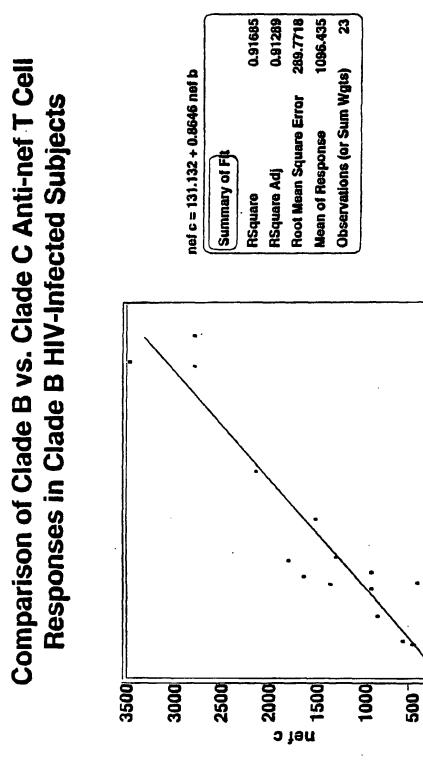


FIGURE 25

nef b



MRKAd5pol MER1062 (MRKAd5 Pre-Adenoviral Vector Containing the IA opt pol Coding Region)

1	CATCATCAAT	AATATACCTT	ATTTTGGATT	GAAGCCAATA	TGATAATGAG
	GTAGTAGTTA	TTATATGGAA	TAAAACCTAA	CTTCGGTTAT	ACTATTACTC
51	GGGGTGGAGT	TTGTGACGTG	GCGCGGGGCG	TGGGAACGGG	GCGGGTGACG
	CCCCACCTCA	AACACTGCAC	CGCGCCCCGC	ACCCTTGCCC	CGCCCACTGC
101			GATGTTGCAA		
	ATCATCACAC	CGCCTTCACA	CTACAACGTT	CACACCGCCT	TGTGTACATT
151	CCCACCCATC	MCCC3 3 3 3 CM	GACGTTTTTG	COCOCCCCC	CTCTT C A C A C
191			CTGCAAAAAC		
	CGC1GCC1AC	ACCULTION	CIGCIDIAN	Characterist	CACAIGIGIC
201	GAAGTGACAA	TTTTCGCGCG	GTTTTAGGCG	GATGTTGTAG	TAAATTTGGG
	CTTCACTGTT	AAAAGCGCGC	CAAAATCCGC	CTACAACATC	ATTTAAACCC
251	CGTAACCGAG	TAAGATTTGG	CCATTTTCGC	GGGAAAACTG	AATAAGAGGA
	GCATTGGCTC	ATTCTAAACC	GGTAAAAGCG	CCCTTTTGAC	TTATTCTCCT
301			GTGTTACTCA		
	TCACTTTAGA	CTTATTAAAA	CACAATGAGT	ATCGCGCATT	ATAAACAGAT
351	ccccccccc	CACTOTCACC	GTTTACGTGG	AGACTICGCCC	ACCINCIPATION
JJ1			CAAATGCACC		
			0.22.1.001.00	1010100000	TCC/IC/DDDD
401	CTCAGGTGTT	TTCCGCGTTC	CGGGTCAAAG	TTGGCGTTTT	ATTATTATAG
	GAGTCCACAA	AAGGCGCAAG	GCCCAGTTTC	AACCGCAAAA	TAATAATATC
451	GCGGCCGCGA	TCCATTGCAT	ACGTTGTATC	CATATCATAA	TATGTACATT
	CGCCGGCGCT	AGGTAACGTA	TGCAACATAG	GTATAGTATT	ATACATGTAA
501			ATTACCGCCA		
	ATATAACCGA	GTACAGGTTG	TAATGGCGGT	ACAACTGTAA	CTAATAACTG
551	тасттаттаа	предрадера	TTACGGGGTC	בררב <u>ה</u> דרבר	AGCCCATATA
			AATGCCCCAG		
601	TGGAGTTCCG	CGTTACATAA	CTTACGGTAA	ATGGCCCGCC	TGGCTGACCG
	ACCTCAAGGC	GCAATGTATT	GAATGCCATT	TACCGGGCGG	ACCGACTGGC
651	• • • • • • • • • • • • • • • • • • • •		GACGTCAATA		
	GGGTTGCTGG	GGGCGGGTAA	CTGCAGTTAT	TACTGCATAC	AAGGGTATCA
701	******		ATTGACGTCA	NTCCCTCCNC	MAMMAN CCCM
701			TAACTGCAGT		
•	1100001171	CCCIONNAGO	IAAC I GCAGI	INCCCACCIC	AIAAAIGCCA
751	AAACTGCCCA	CTTGGCAGTA	CATCAAGTGT	ATCATATGCC	AAGTACGCCC
	TTTGACGGGT				
801	CCTATTGACG	TCAATGACGG	TAAATGGCCC	GCCTGGCATT	ATGCCCAGTA
	GGATAACTGC	AGTTACTGCC	ATTTACCGGG	CGGACCGTAA	TACGGGTCAT
851	CATGACCTTA				
	GTACTGGAAT	ACCCTGAAAG	GATGAACCGT	CATGTAGATG	CATAATCAGT

7 i jure 26A

901	TCGCTATTAC AGCGATAATG	GTACCACTAC	CGCTTTTGGC GCCAAAACCG	AGTACATCAA TCATGTAGTT	TGGGCG LA ACCCGCACCT
951	TAGCGGTTTG ATCGCCAAAC	ACTCACGGGG TGAGTGCCCC	ATTTCCAAGT TAAAGGTTCA	CTCCACCCCA GAGGTGGGGT	TTGACGTCAA AACTGCAGTT
1001			AAAATCAACG TTTTAGTTGC		
1051	TGTTGAGGCG	GGGTAACTGC	CAAATGGGCG GTTTACCCGC	CATCCGCACA	TGCCACCCTC
1101	CAGATATATT	CGTCTCGAGC	TTTAGTGAAC AAATCACTTG	GCAGTCTAGC	GGACCTCTGC
1151	GGTAGGTGCG	ACAAAACTGG	TCCATAGAAG AGGTATCTTC	TGTGGCCCTG	GCTAGGTCGG
1201	AGGCGCCGGC	CCTTGCCACG	ATTGGAACGC TAACCTTGCG	CCTAAGGGGC	ACGGTTCTCA
1251	CTCTAGATGG	TACCGGGGGT	TCTCCCCCAT AGAGGGGGTA	ACTCTGACAC	GGACACTTCG
1301	ACTTCGGACC	GTACCTACCG	CCCAAGGTGA GGGTTCCACT	TCGTCACCGG	GGACTGACTC
1351	CTCTTCTAGT	TCCGGGACCA	GGAAATCTGC CCTTTAGACG	TGACTCTACC	TCTTCCTCCC
1401	GTTTTAGAGG	TTCTAACCGG	CCGAGAACCC GGCTCTTGGG	GATGTTGTGG	GGACACAAAC
1451	GGTAGTTCTT	CTTCCTGAGG	ACCAAGTGGA TGGTTCACCT	CCTTCGACCA	CCTGAAGTCC
1501	CTCGACTTGT	TCTCCTGGGT	GGACTTCTGG CCTGAAGACC	CTCCACGTCG	ACCCGTAGGG
1551	GGTGGGGCGA	CCGGACTTCT	AGAAGAAGTC TCTTCTTCAG	ACACTGACAC	GACCGACACC
1601	CCCTACGGAT	GAAGAGACAC	CCCCTGGATG GGGGACCTAC	TCCTGAAGTC	CTTCATGTGA
	CGGAAGTGGT	AGGGGAGGTA	GTTGTTACTC	TGGGGACCGT	
	CATGTTACAC	GACGGGGTCC	CGACCTTCCC	GAGGGGACGG	ATCTTCCAGT TAGAAGGTCA
1751	CCTCCATGAC GGAGGTACTG	CAAGATCCTG GTTCTAGGAC	GAGCCCTTCA CTCGGGAAGT	GGAAGCAGAA CCTTCGTCTT	CCCTGACATT GGGACTGTAA
1801	GTGATCTACC CACTAGATGG	AGTACATGGC TCATGTACCG	TGCCCTGTAT ACGGGACATA	GTGGGCTCTG CACCCGAGAC	ACCTGGAGAT TGGACCTCTA



1851	TGGGCAGCAC ACCCGTCGTG	A CCAAGA TCCTGGTTCT	TTGAGGAGCT AACTCCTCGA	GAGGCAGCAC CTCCGTCGTG	CTGCTG T GACGACTCCA
1901		CACCCCTGAC GTGGGGACTG			
1951		ATGAGCTGCA TACTCGACGT			
2001		AAGGACTCCT TTCCTGAGGA			
2051		CTGGGCCTCC GACCCGGAGG			
2101		TGCTGAGGGG ACGACTCCCC			
2151		GCTGAGCTGG CGACTCGACC			
2201		TGGGGTGTAC ACCCCACATG			
2251		AGGGCCAGGG TCCCGGTCCC			
2301		CTGAAGACTG GACTTCTGAC			
2351		GAAGCAGCTG CTTCGTCGAC			
2401		TCTGGGGCAA AGACCCCGTT			
2451		GAGACCTGGT CTCTGGACCA			
2501		GTTTGTGAAC CAAACACTTG			
2551	GACCTCTTCC	AGCCCATTGT TCGGGTAACA	CCCCCGACTC	TGGAAGATAC	ACCGACCCCG
2601	TGCCAACAGG ACGGTTGTCC	GAGACCAAGC CTCTGGTTCG	TGGGCAAGGC ACCCGTTCCG	TGGCTATGTG ACCGATACAC	ACCAACAGGG TGGTTGTCCC
2651	GCAGGCAGAA CGTCCGTCTT	GGTGGTGACC CCACCACTGG	CTGACTGACA GACTGACTGT	CCACCAACCA GCTGGTTGGT	GAAGACTGCC CTTCTGACGG
2701	CTCCAGGCCA GAGGTCCGGT				AGGTGAACAT TCCACTTGTA
2751	TGTGACTGCC ACACTGACGG	TCCCAGTATG AGGGTCATAC	CCCTGGGCAT GGGACCCGTA	CATCCAGGCC GTAGGTCCGG	CAGCCTGATC GTCGGACTAG

Figure 26 C

2801	AGTCTGAGTC	T CTGGTG	AACCAGATCA	TTGAGCAGCT	GATCAA G
	TCAGACTCAG	ACTCGACCAC	TTGGTCTAGT	AACTCGTCGA	CTAGTTCTTC
2851	GAGAAGGTGT	ACCTGGCCTG	GGTGCCTGCC	CACAAGGGCA	TTGGGGGCAA
	CTCTTCCACA	TGGACCGGAC	CCACGGACGG	GTGTTCCCGT	AACCCCCGTT
2901	TGAGCAGGTG	GACAAGCTGG	TGTCTGCTGG	CATCAGGAAG	GTGCTGTTCC
	ACTCGTCCAC	CTGTTCGACC	ACAGACGACC	GTAGTCCTTC	CACGACAAGG
2951	TGGATGGCAT	TGACAAGGCC	CAGGATGAGC	ATGAGAAGTA	CCACTCCAAC
	ACCTACCGTA	ACTGTTCCGG	GTCCTACTCG	TACTCTTCAT	GGTGAGGTTG
3001	TGGAGGGCTA	TGGCCTCTGA	CTTCAACCTG	CCCCCTGTGG	TGGCTAAGGA
	ACCTCCCGAT	ACCGGAGACT	GAAGTTGGAC	GGGGGACACC	ACCGATTCCT
3051	GATTGTGGCC	TCCTGTGACA	AGTGCCAGCT	GAAGGGGGAG	GCCATGCATG
	CTAACACCGG	AGGACACTGT	TCACGGTCGA	CTTCCCCCTC	CGGTACGTAC
3101	GGCAGGTGGA	CTGCTCCCCT	GGCATCTGGC	AGCTGGCCTG	CACCCACCTG
	CCGTCCACCT	GACGAGGGGA	CCGTAGACCG	TCGACCGGAC	GTGGGTGGAC
3151	GAGGGCAAGG	TGATCCTGGT	GGCTGTGCAT	GTGGCCTCCG	GCTACATTGA
	CTCCCGTTCC	ACTAGGACCA	CCGACACGTA	CACCGGAGGC	CGATGTAACT
3201	GGCTGAGGTG	ATCCCTGCTG	AGACAGGCCA	GGAGACTGCC	TACTTCCTGC
	CCGACTCCAC	TAGGGACGAC	TCTGTCCGGT	CCTCTGACGG	ATGAAGGACG
3251	TGAAGCTGGC	TGGCAGGTGG	CCTGTGAAGA	CCATCCACAC	TGCCAATGGC
	ACTTCGACCG	ACCGTCCACC	GGACACTTCT	GGTAGGTGTG	ACGGTTACCG
3301	TCCAACTTCA	CTGGGGCCAC	AGTGAGGGCT	GCCTGCTGGT	GGGCTGGCAT
	AGGTTGAAGT	GACCCCGGTG	TCACTCCCGA	CGGACGACCA	CCCGACCGTA
3351	CAAGCAGGAG	TTTGGCATCC	CCTACAACCC	CCAGTCCCAG	GGGGTGGTGG
	GTTCGTCCTC	AAACCGTAGG	GGATGTTGGG	GGTCAGGGTC	CCCCACCACC
3401	CCTCCATGAA	CAAGGAGCTG	AAGAAGATCA	TTGGGCAGGT	GAGGGACCAG
	GGAGGTACTT	GTTCCTCGAC	TTCTTCTAGT	AACCCGTCCA	CTCCCTGGTC
3451	GCTGAGCACC	TGAAGACAGC	TGTGCAGATG	GCTGTGTTCA	TCCACAACTT
	CGACTCGTGG	ACTTCTGTCG	ACACGTCTAC	CGACACAAGT	AGGTGTTGAA
3501	CAAGAGGAAG	GGGGGCATCG	GGGGCTACTC	CGCTGGGGAG	AGGATTGTGG
	GTTCTCCTTC	CCCCCGTAGC	CCCCGATGAG	GCGACCCCTC	TCCTAACACC
3551	ACATCATTGC	CACAGACATC	CAGACCAAGG	AGCTCCAGAA	GCAGATCACC
	TGTAGTAACG	GTGTCTGTAG	GTCTGGTTCC	TCGAGGTCTT	CGTCTAGTGG
3601	AAGATCCAGA	ACTTCAGGGT	GTACTACAGG	GACTCCAGGA	ACCCCCTGTG
	TTCTAGGTCT	TGAAGTCCCA	CATGATGTCC	CTGAGGTCCT	TGGGGGACAC
3651	GAAGGGCCCT	GCCAAGCTGC	TGTGGAAGGG	GGAGGGGGCT	GTGGTGATCC
	CTTCCCGGGA	CGGTTCGACG	ACACCTTCCC	CCTCCCCGA	CACCACTAGG
3701	AGGACAACTC	TGACATCAAG	GTGGTGCCCA	GGAGGAAGGC	CAAGATCATC
	TCCTGTTGAG	ACTGTAGTTC	CACCACGGGT	CCTCCTTCCG	GTTCTAGTAG

Figure 26 D

3751	AGGGACTATG	AGCAGAT	GGCTGGGGAT	GACTGTGTĞĞ	CCTCCA CA
	TCCCTGATAC	CC.TCGTCTA	CCGACCCCTA	CTGACACACC	GGAGGT GT
3801	GGATGAGGAC	TAAAGCCCGG	GCAGATCTGC	TGTGCCTTCT	AGTTGCCAGC
	CCTACTCCTG	ATTTCGGGCC	CGTCTAGACG	ACACGGAAGA	TCAACGGTCG
3851	CATCTGTTGT	TTGCCCCTCC	CCCGTGCCTT	CCTTGACCCT	GGAAGGTGCC
	GTAGACAACA	AACGGGGAGG	GGGCACGGAA	GGAACTGGGA	CCTTCCACGG
3901	ACTCCCACTG	TCCTTTCCTA	ATAAAATGAG	GAAATTGCAT	CGCATTGTCT
	TGAGGGTGAC	AGGAAAGGAT	TATTTTACTC	CTTTAACGTA	GCGTAACAGA
3951	GAGTAGGTGT	CATTCTATTC	TGGGGGGTGG	GGTGGGGCAG	GACAGCAAGG
	CTCATCCACA	GTAAGATAAG	ACCCCCCACC	CCACCCGTC	CTGTCGTTCC
4001	GGGAGGATTG	GGAAGACAAT	AGCAGGCATG	CTGGGGATGC	GGTGGGCTCT
	CCCTCCTAAC	CCTTCTGTTA	TCGTCCGTAC	GACCCCTACG	CCACCCGAGA
4051	ATGGCCGATC TACCGGCTAG	GGCGCGCCGT	ACTGAAATGT TGACTTTACA	GTGGGCGTGG CACCCGCACC	CTTAAGGGTG GAATTCCCAC
4101	GGAAAGAATA	TATAAGGTGG	GGGTCTTATG	TAGTTTTGTA	TCTGTTTTGC
	CCTTTCTTAT	ATATTCCACC	CCCAGAATAC	ATCAAAACAT	AGACAAAACG
4151	AGCAGCCGCC	GCCGCCATGA	GCACCAACTC	GTTTGATGGA	AGCATTGTGA
	TCGTCGGCGG	CGGCGGTACT	CGTGGTTGAG	CAAACTACCT	TCGTAACACT
4201	GCTCATATTT	GACAACGCGC	ATGCCCCCAT	GGGCCGGGT	GCGTCAGAAT
	.CGAGTATAAA	CTGTTGCGCG	TACGGGGGTA	CCCGGCCCCA	CGCAGTCTTA
4251	GTGATGGGCT	CCAGCATTGA	TGGTCGCCCC	GTCCTGCCCG	CAAACTCTAC
	CACTACCCGA	GGTCGTAACT	ACCAGCGGGG	CAGGACGGGC	GTTTGAGATG
4301	TACCTTGACC	TACGAGACCG	TGTCTGGAAC	GCCGTTGGAG	ACTGCAGCCT
	ATGGAACTGG	ATGCTCTGGC	ACAGACCTTG	CGGCAACCTC	TGACGTCGGA
4351	CCCCCCCCCC	TTCAGCCGCT AAGTCGGCGA	GCAGCCACCG CGTCGGTGGC	CCCGCGGGAT GGGCGCCCTA	TGTGACTGAC ACACTGACTG
4401	TTTGCTTTCC	TGAGCCCGCT	TGCAAACAGT	GCAGCTTCCC	GTTCATCCGC
	AAACGAAAGG	ACTCGGGCGA	ACGTTTGTCA	CGTCGAAGGG	CAAGTAGGCG
4451	CCGCGATGAC	AAGTTGACGG	CTCTTTTGGC	ACAATTGGAT	TCTTTGACCC
	GGCGCTACTG	TTCAACTGCC	GAGAAAACCG	TGTTAACCTA	AGAAACTGGG
4501	GGGAACTTAA	TGTCGTTTCT	CAGCAGCTGT	TGGATCTGCG	CCAGCAGGTT
	CCCTTGAATT	ACAGCAAAGA	GTCGTCGACA	ACCTAGACGC	GGTCGTCCAA
4551	TCTGCCCTGA	AGGCTTCCTC	CCCTCCCAAT	GCGGTTTAAA	ACATAAATAA
	AGACGGGACT	TCCGAAGGAG	GGGAGGGTTA	CGCCAAATTT	TGTATTTATT
4601	AAAACCAGAC	TCTGTTTGGA	TTTGGATCAA	GCAAGTGTCT	TGCTGTCTTT
	TTTTGGTCTG	AGACAAACCT	AAACCTAGTT	CGTTCACAGA	ACGACAGAAA
4651	ATTTAGGGGT	TTTGCGCGCGC	CGGTAGGCCC	GGGACCAGCG	GTCTCGGTCG
	TAAATCCCCA	AAACGCGCGC	GCCATCCGGG	CCCTGGTCGC	CAGAGCCAGC

Figure 26E

4701	TTGAGGGTCC AACTCCCAGG	TCTGTATTTT ATAAAA	TTCCAGGACG AAGGTCCTGC	TGGTAAAGGT ACCATTTCCA	-GACTCTCGAT CTGAGA A
4751		ATGGGCATAA TACCCGTATT			
4801		ATGCTGCGGG TACGACGCCC			
4851		CGTGGTGCCT GCACCACGGA			
4901	GTCCCCGTCC	CCCTTGGTGT GGGAACCACA	TTCACAAATG	TTTCGCCAAT	TCGACCCTAC
4951		TGGGGATATG ACCCCTATAC			
5001		CAGCCATATC GTCGGTATAG			
5051		TATCCGGTGC ATAGGCCACG			
5101	CTTTACGCAC	GAAGAACTTG CTTCTTGAAC	CTCTGCGGGA	ACACTGGAGG	TTCTAAAAGG
5151	TACGTAAGCA	CCATAATGAT GGTATTACTA	CCGTTACCCG	GGTGCCCGCC	GCCGGACCCG
5201	C'ITCTATAAA	CTGGGATCAC GACCCTAGTG	ATTGCAGTAT	CAACACAAGG	TCCTACTCTA
5251	GCAGTATCCG	CATTTTTACA GTAAAAATGT	TTCGCGCCCG	CCTCCCACGG	TCTGACGCCA
5301	TATTACCAAG	CATCCGGCCC GTAGGCCGGG	TCCCCGCATC	AATGGGAGTG	TCTAAACGTA
5351	AAGGGTGCGA	TTGAGTTCAG AACTCAAGTC	TACCCCCCTA	GTACAGATGG	ACGCCCCCCT
5401		CCAAAGGCCC	CATCCCCTCT	AGTCGACCCT	TCTTTCGTCC
		CGACGCTGAA	TGGCGTCGGC	CACCCGGGCA	TTTAGTGTGG
		ACGTTGACCA	TCAATTCTCT	CGACGTCGAC	GGCAGTAGGG
		CCGGTGAAGC	AATTCGTACA	GGGACTGAGC	GTACAAAAGG
5601	CTGACCAAAT GACTGGTTTA				GCAGTTCTTG CGTCAAGAAC

Figure 26 F

5651	CAAGGAAGCA GTTCCTTCGT			ACCGTCCGCC TGGCAGGCGG	
5701				GGTCCCACAG CCAGGGTGTC	
5751	TGCTCTACGG	CATCTCGATC	CAGCATATCT	CCTCGTTTCG	CGGGTTGGGG
	ACGAGATGCC	GTAGAGCTAG	GTCGTATAGA	GGAGCAAAGC	GCCCAACCCC
5801	CGGCTTTCGC	TGTACGGCAG	TAGTCGGTGC	TCGTCCAGAC	GGGCCAGGGT
	GCCGAAAGCG	ACATGCCGTC	ATCAGCCACG	AGCAGGTCTG	CCCGGTCCCA
5851	CATGTCTTTC	CACGGGCGCA	GGGTCCTCGT	CAGCGTAGTC	TGGGTCACGG
	GTACAGAAAG	GTGCCCGCGT	CCCAGGAGCA	GTCGCATCAG	ACCCAGTGCC
5901	TGAAGGGGTG	CGCTCCGGGC	TGCGCGCTGG	CCAGGGTGCG	CTTGAGGCTG
	ACTTCCCCAC	GCGAGGCCCG	ACGCGCGACC	GGTCCCACGC	GAACTCCGAC
5951				TCGCCCTGCG AGCGGGACGC	
6001	GTAGCATTTG	ACCATGGTGT	CATAGTCCAG	CCCCTCCGCG	GCGTGGCCCT
	CATCGTAAAC	TGGTACCACA	GTATCAGGTC	GGGGAGGCGC	CGCACCGGGA
6051	TGGCGCGCAG	CTTGCCCTTG	GAGGAGGCGC	CGCACGAGGG	GCAGTGCAGA
	ACCGCGCGTC	GAACGGGAAC	CTCCTCCGCG	GCGTGCTCCC	CGTCACGTCT
6101	CTTTTGAGGG	CGTAGAGCTT	GGGCGCGAGA	AATACCGATT	CCGGGGAGTA
	GAAAACTCCC	GCATCTCGAA	CCCGCGCTCT	TTATGGCTAA	GGCCCCTCAT
6151				CTCGCATTCC GAGCGTAAGG	
6201	TGAGCTCTGG	CCGTTCGGGG	TCAAAAACCA	GGTTTCCCCC	ATGCTTTTTG
	ACTCGAGACC	GGCAAGCCCC	AGTTTTTGGT	CCAAAGGGGG	TACGAAAAAC
6251				CGGTGTCCAC GCCACAGGTG	
6301				CTTGAGAGGC GAACTCTCCG	
6351				ACTCGGACCA TGAGCCTGGT	CTCTGAGACA GAGACTCTGT
6401	AAGGCTCGCG	TCCAGGCCAG	CACGAAGGAG	GCTAAGTGGG	AGGGGTAGCG
	TTCCGAGCGC	AGGTCCGGTC	GTGCTTCCTC	CGATTCACCC	TCCCCATCGC
6451	GTCGTTGTCC	ACTAGGGGGT	CCACTCGCTC	CAGGGTGTGA	AGACACATGT
	CAGCAACAGG	TGATCCCCCA	GGTGAGCGAG	GTCCCACACT	TCTGTGTACA
6501	CGCCCTCTTC	GGCATCAAGG	AAGGTGATTG	GTTTGTAGGT	GTAGGCCACG
	GCGGGAGAAG	CCGTAGTTCC	TTCCACTAAC	CAAACATCCA	CATCCGGTGC
6551					GGGCGCGTTC CCCGCGCAAG

Figure 266

6601	GTCCTCACTC CAGGAGTGAG	TCTTCCGCAT AGGCGTA	CGCTGTCTGC GCGACAGACG	CAGGGCCAGG CTCCCGGTCG	ACAACO C
6651	AGTACTCCCT TCATGAGGGA	CTGAAAAGCG GACTTTTCGC	GGCATGACTT CCGTACTGAA	CTGCGCTAAG GACGCGATTC	ATTGTCAGTT TAACAGTCAA
6701	TCCAAAAACG AGGTTTTTGC	AGGAGGATTT TCCTCCTAAA	GATATTCACC CTATAAGTGG	TGGCCCGCGG ACCGGGCGCC	TGATGCCTTT ACTACGGAAA
6751	GAGGGTGGCC CTCCCACCGG	GCATCCATCT CGTAGGTAGA	GGTCAGAAAA CCAGTCTTTT	GACAATCTTT CTGTTAGAAA	TTGTTGTCAA AACAACAGTT
6801	CGAACCACCG	TTTGCTGGGC	ATCTCCCGCA	TGGACAGCAA ACCTGTCGTT	GAACCGCTAC
6851	CTCGCGTCCC	AAACCAAAAA	CAGCGCTAGC	GCGCGCTCCT CGCGCGAGGA	ACCGGCGCTA
6901	CAAATCGACG	TGCATAAGCG	CCCCTTCCCT	CCGCCATTCG GGCGGTAAGC	CCTTTCTGCC
6951	ACCACGCGAG	CAGCCCGTGG	TCCACGTGCG	GCCAACCGCG CGGTTGGCGC	CAACACGTCC
7001	CACTGTTCCA	GTTGCGACCA	CCGATGGAGA	CCGCGTAGGC GGCGCATCCG	CGAGCAACCA
7051	GGTĊGTCTCC	GCCGGCGGGA	ACGCGCTCGT	GAATGGCGGT CTTACCGCCA	TCCCCCAGAT
7101	CGACGCAGAG	CAGGCCCCCC	AGACGCAGGT	CGGTAAAGAC GCCATTTCTG	GGGCCCGTCG
7151	TCCGCGCGCA	GCTTCATCAG	ATAGAACGTA	CCTTGCAAGT GGAACGTTCA	GATCGCGGAC
7201	GACGGTACGC	GCCGCCGTT	CGCGCGCGAG	GTATGGGTTG CATACCCAAC	TCACCCCCTG
7251	GGGTACCGTA	CCCCACCCAC	TCGCGCCTCC	CGTACATGCC GCATGTACGG	CGTTTACAGC
7301	ATTTGCATCT	CCCCGAGAGA	CTCATAAGGT	AGATATGTAG TCTATACATC	CCATCGTAGA
		TACGACCGCG	CGTGCATTAG	CATATCAAGC	ACGCTCCCTC
		CCCTGGCTCC	AACGATGCCC	GCCCGACGAG	ACGAGCCTTC
	TGATAGACGG	ACTTCTACCG	TACACTCAAC	CTACTATACC	TTGGACGCTG AACCTGCGAC
7501	GAAGACGTTG CTTCTGCAAC	AAGCTGGCGT TTCGACCGCA	CTGTGAGACC GACACTCTGG	TACCGCGTCA ATGGCGCAGT	CGCACGAAGG GCGTGCTTCC

Figure 26 H

7551	AGGCGTAGGA TCCGCATCCT	GCGCAGC CAGCGCGTCG	TTGTTGACCA AACAACTGGT	GCTCGGCGGT CGAGCCGCCA	GACCTG G CTGGACGTGC
7601		AGTAGTCCAG TCATCAGGTC			
7651		TTCCACAGCT AAGGTGTCGA			
7701		TTGGATCGGA AACCTAGCCT			
7751	AGCATGTAGA TCGTACATCT	ACTGGTTGAC TGACCAACTG	GGCCTGGTAG CCGGACCATC	GCGCAGCATC CGCGTCGTAG	·CCTTTTCTAC GGAAAAGATG
7801	GGGTAGCGCG CCCATCGCGC	TATGCCTGCG ATACGGACGC	CGGCCTTCCG GCCGGAAGGC	CACCGACGTC CTCGCTCCAC	TGGGTGAGCG ACCCACTCGC
7851		CCTGACCATG GGACTGGTAC			
7901		CGCCCTGCTC GCGGGACGAG			
7951		GGCAGGGCGA CCGTCCCGCT			
8001	CGCGAGGCAT GCGCTCCGTA	AAAGTTGCGT TTTCAACGCA	GTGATGCGGA CACTACGCCT	AGGGTCCCGG TCCCAGGGCC	CACCTCGGAA GTGGAGCCTT
8051		TTACCTGGGC AATGGACCCG			
8101		ACAATGTAAA TGTTACATTT			
8151		TTTAAGTTCC AAATTCAAGG			
8201	CCGTGCTCTG GGCACGAGAC	AAAGGGCCCA TTTCCCGGGT	GTCTGCAAGA CAGACGTTCT	TGAGGGTTGG ACTCCCAACC	AAGCGACGAA TTCGCTGCTT
8251		AGGTCACGGG TCCAGTGCCC			
8301	TCCTAAACTG AGGATTTGAC	GCGACCTATG CCCTGGATAC	GCCATTTTTT CGGTAAAAA	CTGGGGTGAT GACCCCACTA	GCAGTAGAAG CGTCATCTTC
8351	GTAAGCGGGT CATTCGCCCA	CTTGTTCCCA GAACAAGGGT	GCGGTCCCAT CGCCAGGGTA	CCAAGGTTCG GGTTCCAAGC	CGGCTAGGTC GCCGATCCAG
8401	TCGCGCGCCA AGCGCGCCGT	GTCACTAGAG CAGTGATCTC			
B451					ATAGGTCTCT TATCCAGAGA

Figure 26I

8501	ACATCGTAGG TGTAGCATCC	TAAAGAG ACTGTTTCTC	ACGCTCGGTG TGCGAGCCAC	CGAGGATGCG GCTCCTACGC	AGCCGA G TCGGCTAGCC
8551	CTTCTTGACC	TAGAGGGCGG	ACCAATTGGA TGGTTAACCT	CCTCACCGAT	AACTACACCA
8601	CTTTCATCTT	CAGGGACGCT	CGGGCCGAAC GCCCGGCTTG	TGAGCACGAC	CGAAAACATT
8651	TTTGCACGCG	TCATGACCGT	GCGGTGCACG CGCCACGTGC	CCGACATGTA	GGACGTGCTC
8701	CAACTGGACT	GCTGGCGCGT	CAAGGAAGCA GTTCCTTCGT	CTCACCCTTA	AACTCGGGGA
8751	GCGGACCGCC	CAAACCGACC	TGGTCTTCTA ACCAGAAGAT	GAAGCCGACG	AACAGGAACT
8801	GGCAGACCGA	CGAGCTCCCC	AGTTACGGTG TCAATGCCAC	CTAGCCTGGT	GGTGCGGCGC
8851	GCTCGGGTTT	CAGGTCTACA	CCGCGCGCGC	GCCAGCCTCG	AACTACTGTT
8901	GTAGCGCGTC	TACCCTCGAC	TCCATGGTCT AGGTACCAGA	CCTCGAGGGC	GCCGCAGTCC
8951	AGTCCGCCCT	CGAGGACGTC	GTTTACCTCG CAAATGGAGC	GTATCTGCCC	AGTCCCGCGC
9001	CCGATCTAGG	TCCACTATGG	TAATTTCCAG ATTAAAGGTC	CCCGACCAAC	CACCGCCGCA
9051	GCTACCGAAC	GTTCTCCGGC	CATCCCCGCG	CGCGCTGATG	CCATGGCGCG
9101	CCGCCCGCCA	CCCGGCGCCC	GGTGTCCTTG	CTACTACGTA	GATTTTCGCC
9151	ACTGCGCCCG	CTCGGGGGCC	AGGTAGGGGG	CCGAGGCCTG	GGCGGCCCTC
9201	TCCCCCGTCC	CCGTGCAGCC	GCGGCGCGCG	CCCGTCCTCG	
	GCGCATCCAA	CGACCGCTTG	CGCTGCTGCG	CCGCCAACTA	CTCCTGAATC GAGGACTTAG
	ACCGCGGAGA	CGCACTTCTG	CTGCCCGGGC	CACTCGAACT	ACCTGAAAGA
	CTCAAGCTGT	CTTAGTTAAA	GCCACAGCAA	CTGCCGCCGG	TGGCGCAAAA ACCGCGTTTT
9401	TCTCCTGCAC AGAGGACGTG	GTCTCCTGAG GCAGAGGACTC	TIGTCTTGAT	AGGCGATCTC TCCGCTAGAG	GGCCATGAAC

Figure 26 J

9451		CTCCTG GAAGGAGGAC			
9501		TCGTTGGAAA AGCAACCTTT			
9551		GTTCCAGACG CAAGGTCTGC			
9601		TGACCACCTG ACTGGTGGAC			
9651		TTTCGCAGGC AAAGCGTCCG			
9701		CACGAAGAAG GIGCTICTIC			
9751	TTGATATCCC AACTATAGGG	CCAAGGCCTC GGTTCCGGAG			
9801		AAAAACTGGG TTTTTGACCC			
9851		GATGAGCTCG CTACTCGAGC			
9901		CCTCTTCTTC GGAGAAGAAG			
9951		TCTTCTGGCG AGAAGACCGC			
10001		CGGGAGGCGG GCCCTCCGCC			
10051		TGGTCTCGGT ACCAGAGCCA			
10101		CCGCCCGTCA GGCGGGCAGT			
10151		GGATACGGCG CCTATGCCGC			
10201	GGTACTCCGC CCATGAGGCG	CGCCGAGGGA GCGGCTCCCT	CCTGAGCGAG GGACTCGCTC	TCCGCATCGA AGGCGTAGCT	CCGGATCGGA GGCCTAGCCT
10251	AAACCTCTCG TTTGGAGAGC	AGAAAGGCGT TCTTTCCGCA	CTAACCAGTC GATTGGTCAG	ACAGTCGCAA TGTCAGCGTT	GGTAGGCTGA CCATCCGACT
10301	GCACCGTGGC CGTGGCACCG	GGGCGGCAGC CCCGCCGTCG	CCCGCCGCCA	CGGGGTTGTT GCCCCAACAA	TCTGGCGGAG AGACCGCCTC
10351	GTGCTGCTGA CACGACGACT	TGATGTAATT ACTACATTAA	AAAGTAGGCG TTTCATCCGC	GTCTTGAGAC CAGAACTCTG	GGCGGATGGT CCGCCTACCA

Figure 26 K

10401	CGACAGAAGC GCTGTCTTCG	A TGTCCT TO TACAGGA	TGGGTCCGGC ACCCAGGCCG	CTGCTGAATG GACGACTTAC	CCCACC A
10451	CGGCCATGCC GCCGGTACGG	CCAGGCTTCG GGTCCGAAGC	TTTTGACATC AAAACTGTAG	GGCGCAGGTC CCGCGTCCAG	TTTGTAGTAG AAACATCATC
10501	TCTTGCATGA AGAACGTACT	GCCTTTCTAC CGGAAAGATG	CGGCACTTCT GCCGTGAAGA	TCTTCTCCTT AGAAGAGGAA	CCTCTTGTCC GGAGAACAGG
10551	ACGTAGAGAA	CGTAGATAGC	GACGCCGCCG	CCGCCTCAAA	CCGGCATCCA
10601	CCGCGGGAGA	TCCTCCCATG AGGAGGGTAC	GCACACTGGG	GCTTCGGGGA	GTAGCCGACT
10651	TCGTCCCGAT	GGTCGGCGAC CCAGCCGCTG	TTGCGCGAGC	CGATTATACC	GGACGACGTG
10701	GACGCACTCC	GTAGACTGGA CATCTGACCT	TCAGTAGGTA	CAGGTGTTTC	GCCACCATAC
10751	GCGGGCACAA	GATGGTGTAA CTACCACATT	CACGTCAACC	GGTATTGCCT	GGTCAATTGC
10801	CAGACCACTG	CCGGCTGCGA GGCCGACGCT	CTCGAGCCAC	ATGGACTCTG	CGCTCATTCG
10851	GGAGCTCAGT	AATACGTAGT TTATGCATCA	GCAACGTTCA	GGCGTGGTCC	ATGACCATAG
10901	GGTGGTTTTT	GTGCGGCGGC CACGCCGCCG	CCGACCGCCA	TCTCCCCGGT	CGEATCCCAC
10951	CGGCCCCGAG	CGGGGGGGAG GCCCCGGTC	TAGAAGGTTG	TATTCCGCTA	CTATAGGCAT
11001	CTACATGGAC	GACATCCAGG CTGTAGGTCC	ACTACGGCCG	CCGCCACCAC	CTCCGCGCGC
11051	CTTTCAGCGC	GACGCGGTTC CTGCGCCAAG	GTCTACAACG	CGTCGCCGTT	TTTCACGAGG
11101	TACCAGCCCT	GCGAGACCGG	CCAGTCCGCG	CGCGTTAGCA	
		TTCCTCTCGG	ACATTCGCCC	GTGAGAAGGC	ACCAGACCAC
	_	GTTCCCATAG	TACCGCCTGC	TGGCCCCAAG	CTCGGGGCAT
		GCGGCACTAG	GTACGCCAAT	GGCGGGCGCA	CAGCTTGGGT
11301	GGTGTGCGAC CCACACGCTG	GTCAGACAAC CAGTCTGTTG	GGGGGAGTGC CCCCCTCACG	TCCTTTTGGC AGGAAAACCG	TTCCTTCCAG AAGGAAGGTC

Figure 26L

11351	CGCGCCGCCGC	T FGCGCTA ACGACGCGAT	GCTTTTTTGG CGAAAAAACC	CCACTGGCCG GGTGACCGGC	CGCGCA TT GCGCGTCCA
11401	AAGCGGTTAG TTCGCCAATC			AGTGGCTCGC TCACCGAGCG	
11451				GGGACCCCCG CCCTGGGGGC	
11501				TTGCCTCCCC AACGGAGGGG	
11551				GACGAGCCCC CTGCTCGGGG	
11601				GCGCCCCCT	
11651	GGCAAGAGCA CCGTTCTCGT			GGGCACCCTC CCCGTGGGAG	
11701				GACGCGGCAG CTGCGCCGTC	
11751				CTACCTGGAC GATGGACCTG	
11801				CTCCTGAGCG GAGGACTCGC	
11851				TACGTGCCGC ATGCACGGCG	
11901				GGAGATGCGG CCTCTACGCC	
11951				TGAATCGCGA ACTTAGCGCT	
12001				ACCGGGATTA TGGCCCTAAT	
12051				CGCATACGAG GCGTATGCTC	
12101	ACCAGGAGAT TGGTCCTCTA	TAACTTTCAA ATTGAAAGTT	AAAAGCTTTA TTTTCGAAAT	ACAACCACGT TGTTGGTGCA	GCGTACGCTT CGCATGCGAA
12151	GTGGCGCGCG CACCGCGCGC				GGGACTTTGT CCCTGAAACA
12201	AAGCGCGCTG TTCGCGCGAC				GCGCAGCTGT CGCGTCGACA
12251	TCCTTATAGT AGGAATATCA			AGGCATTCAG TCCGTAAGTC	

7 igure 26 M

12301	CTAAACATAG	T GCCCGA	GGGCCGCTGG	CTGCTCGATT	TGATAA TT
	GATTTGTATC	ATCTCGGGCT	CCCGGCGACC	GACGAGCTAA	ACTATTTGTA
12351	CCTGCAGAGC	ATAGTGGTGC	AGGAGCGCAG	CTTGAGCCTG	GCTGACAAGG
	GGACGTCTCG	TATCACCACG	TCCTCGCGTC	GAACTCGGAC	CGACTGTTCC
12401	TGGCCGCCAT	CAACTATTCC	ATGCTTAGCC	TGGGCAAGTT	TTACGCCCGC
	ACCGGCGGTA	GTTGATAAGG	TACGAATCGG	ACCCGTTCAA	AATGCGGGCG
12451	AAGATATACC TTCTATATGG	ATACCCCTTA TATGGGGAAT	CGTTCCCATA GCAAGGGTAT	GACAAGGAGG CTGTTCCTCC	TAAAGATCGA ATTTCTAGCT
12501	GGGGTTCTAC	ATGCGCATGG	CGCTGAAGGT	GCTTACCTTG	AGCGACGACC
	CCCCAAGATG	TACGCGTACC	GCGACTTCCA	CGAATGGAAC	TCGCTGCTGG
12551	ACCCGCAAAT	AGCGTTGCTC	CGCATCCACA GCGTAGGTGT	TCCGGCACTC	GCACTCGGCC
12601	CGGCGCGAGC	TCAGCGACCG AGTCGCTGGC	CGAGCTGATG GCTCGACTAC	CACAGCCTGC GTGTCGGACG	AAAGGGCCCT TTTCCCGGGA
12651	GGCTGGCACG	GGCAGCGGCG	ATAGAGAGGC	CGAGTCCTAC	TTTGACGCGG
	CCGACCGTGC	CCGTCGCCGC	TATCTCTCCG	GCTCAGGATG	AAACTGCGCC
12701	GCGCTGACCT	GCGCTGGGCC	CCAAGCCGAC	GCGCCCTGGA	GGCAGCTGGG
	CGCGACTGGA	CGCGACCCGG	GGTTCGGCTG	CGCGGGACCT	CCGTCGACCC
12751	GCCGGACCTG CGGCCTGGAC	GGCTGGCGGT CCGACCGCCA	GGCACCCGCG CCGTGGGCGC	CGCGCTGGCA	ACGTCGGCGG TGCAGCCGCC
12801	CGTGGAGGAA	TATGACGAGG	ACGATGAGTA	CGAGCCAGAG	GACGGCGAGT
	GCACCTCCTT	ATACTGCTCC	TGCTACTCAT	GCTCGGTCTC	CTGCCGCTCA
12851	ACTAAGCGGT	GATGTTTCTG	ATCAGATGAT	GCAAGACGCA	ACGGACCCGG
	TGATTCGCCA	CTACAAAGAC	TAGTCTACTA	CGTTCTGCGT	TGCCTGGGCC
12901	CGGTGCGGGC	GGCGCTGCAG	AGCCAGCCGT	CCGGCCTTAA	CTCCACGGAC
	GCCACGCCCG	CCGCGACGTC	TCGGTCGGCA	GGCCGGAATT	GAGGTGCCTG
12951	GACTGGCGCC	AGGTCATGGA	CCGCATCATG	TCGCTGACTG	CGCGCAATCC
	CTGACCGCGG	TCCAGTACCT	GGCGTAGTAC	AGCGACTGAC	GCGCGTTAGG
13001	ACTGCGCAAG	GCCGTCGTCG	GCGTCCGGTT	GGCCGAGAGG	
13051	AAGCGGTGGT TTCGCCACCA	CCCGGCGCGC	GCAAACCCCA CGTTTGGGGT	CGCACGAGAA GCGTGCTCTT	GGTGCTGGCG CCACGACCGC
13101	ATCGTAAACG TAGCATTTGC	CGCTGGCCGA	AAACAGGGCC TTTGTCCCGG	ATCCGGCCCG TAGGCCGGGC	ACGAGGCCGG TGCTCCGGCC
13151	CCTGGTCTAC	GACGCGCTGC	TTCAGCGCGT	GGCTCGTTAC	AACAGCGGCA
	GGACCAGATG	CTGCGCGACG	AAGTCGCGCA	CCGAGCAATG	TTGTCGCCGT
13201	ACGTGCAGAC	CAACCTGGAC	CGGCTGGTGG	GGGATGTGCG	CGAGGCCGTG
	TGCACGTCTG	GTTGGACCTG	GCCGACCACC	CCCTACACGC	GCTCCGGCAC

Figure 26 N.

13251	GCGCAGCGTG CGCGTCGCAC	A CGCGCGCA TCGCGCGCGT	GCAGCAGGGC CGTCGTCCCG	AACCTGGGCT TTGGACCCGA	CCATGG C GGTACCAACG
13301			CACAGCCCGC GTGTCGGGCG		
13351			AGCGCACTGC TCGCGTGACG		
13401			GTCTGGGCCA CAGACCCGGT		
13451			TAAACCTGAG ATTTGGACTC		
13501			GCTCCCACAG CGAGGGTGTC		
13551	AGCTTGCTGA TCGAACGACT		GCGCCTGTTG CGCGGACAAC		
13601			CCCGGGACAC GGGCCCTGTG		
13651			GGTCAGGCGC CCAGTCCGCG		
13701			CCGCGCGCTG GGCGCGCGAC		
13751			ACCTGCTGAC TGGACGACTG		
13801			AGCGAGGAGG TCGCTCCTCC		
13851			CCTGATGCGC GGACTACGCG		
13901			GCAACATGGA CGTTGTACCT		
13951			CTAATGGACT GATTACCTGA		
14001	GTGAACCCCG CACTTGGGGC	AGTATTTCAC TCATAAAGTG	CAATGCCATC GTTACGGTAG	TTGAACCCGC AACTTGGGCG	ACTGGCTACC TGACCGATGG
14051	GCCCCTGGT CGGGGGACCA	TTCTACACCG AAGATGTGGC	GGGGATTCGA CCCCTAAGCT	GGTGCCCGAG CCACGGGCTC	GGTAACGATG CCATTGCTAC
14101	GATTCCTCTG CTAAGGAGAC	GGACGACATA CCTGCTGTAT	GACGACAGCG CTGCTGTCGC	TGTTTTCCCC ACAAAAGGGG	GCAACCGCAG CGTTGGCGTC
14151	ACCCTGCTAG TGGGACGATC	AGTTGCAACA TCAACGTTGT	GCGCGAGCAG CGCGCTCGTC	GCAGAGGCGG CGTCTCCGCC	CGCTGCGAAA GCGACGCTTT

7, gure 260

14201	GGAAAGCTTC CCTTTCGAAG	CARAGGCCAA GCCGGTT	GCAGCTTGTC CGTCGAACAG	CGATCTAGGCT GCTAGATCCG	CGACGC G
14251	CGCGGTCAGA	TGCTAGTAGC	CCATTTCCAA	GCTTGATAGG	GTCTCTTACC
	GCGCCAGTCT	ACGATCATCG	GGTAAAGGTT	CGAACTATCC	CAGAGAATGG
14301	AGCACTCGCA	CCACCCGCCC	GCGCCTGCTG	GGCGAGGAGG	AGTACCTAAA
	TCGTGAGCGT	GGTGGGCGGG	CGCGGACGAC	CCGCTCCTCC	TCATGGATTT
14351	CAACTCGCTG	CTGCAGCCGC	AGCGCGAAAA	AAACCTGCCT	CCGGCATTTC
	GTTGAGCGAC	GACGTCGGCG	TCGCGCTTTT	TTTGGACGGA	GGCCGTAAAG
14401	CCAACAACGG	GATAGAGAGC	CTAGTGGACA	AGATGAGTAG	ATGGAAGACG
	GGTTGTTGCC	CTATCTCTCG	GATCACCTGT	TCTACTCATC	TACCTTCTGC
14451	TACGCGCAGG ATGCGCGTCC	AGCACAGGGA TCGTGTCCCT	CGTGCCAGGC GCACGGTCCG	00000000000000000000000000000000000000	CCACCCGTCG GGTGGGCAGC
14501	TCAAAGGCAC	GACCGTCAGC	GGGGTCTGGT	GTGGGAGGAC	GATGACTCGG
	AGTTTCCGTG	CTGGCAGTCG	CCCCAGACCA	CACCCTCCTG	CTACTGAGCC
14551	CAGACGACAG	CAGCGTCCTG	GATTTGGGAG	GGAGTGGCAA	CCCGTTTGCG
	GTCTGCTGTC	GTCGCAGGAC	CTAAACCCTC	CCTCACCGTT	GGGCAAACGC
14601	CACCTTCGCC	CCAGGCTGGG	GAGAATGTTT	TAAAAAAAA	AAAAGCATGA
	GTGGAAGCGG	GGTCCGACCC	CTCTTACAAA	TTTTTTTT	TTTTCGTACT
14651	TGCAAAATAA	AAAACTCACC	AAGGCCATGG	CACCGAGCGT	TGGTTTTCTT
	ACGTTTTATT	TTTTGAGTGG	TTCCGGTACC	GTGGCTCGCA	ACCAAAAGAA
14701	GTATTCCCCT	TAGTATGCGG	CGCGCGGCGA	TGTATGAGGA	AGGTCCTCCT
	CATAAGGGGA	ATCATACGCC	GCGCGCCGCT	ACATACTCCT	TCCAGGAGGA
14751	CCCTCCTACG	AGAGTGTGGT	GAGCGCGGCG	CCAGTGGCGG	CGGCGCTGGG
	GGGAGGATGC	TCTCACACCA	CTCGCGCCGC	GGTCACCGCC	GCCGCGACCC
14801	TTCTCCCTTC	GATGCTCCCC	TGGACCCGCC	GTTTGTGCCT	CCGCGGTACC
	AAGAGGGAAG	CTACGAGGGG	ACCTGGGCGG	CAAACACGGA	GGCGCCATGG
14851	TGCGGCCTAC	CGGGGGGAGA	AACAGCATCC	GTTACTCTGA	GTTGGCACCC
	ACGCCGGATG	GCCCCCTCT	TTGTCGTAGG	CAATGAGACT	CAACCGTGGG
14901	CTATTCGACA	CCACCCGTGT	GTACCTGGTG	GACAACAAGT	CAACGGATGT
	GATAAGCTGT	GGTGGGCACA	CATGGACCAC	CTGTTGTTCA	GTTGCCTACA
14951	GCCATCCCTG	AACTACCAGA	ACGACCACAG	CAACTTTCTG	ACCACGGTCA
	CCGTAGGGAC	TTGATGGTCT	TGCTGGTGTC	GTTGAAAGAC	TGGTGCCAGT
15001	TTCAAAACAA	TGACTACAGC	CCGGGGGAGG	CAAGCACACA	GACCATCAAT
	AAGTTTTGTT	ACTGATGTCG	GGCCCCCTCC	GTTCGTGTGT	CTGGTAGTTA
15051	CTTGACGACC	GGTCGCACTG	GGGCGGCGAC	CTGAAAACCA	TCCTGCATAC
	GAACTGCTGG	CCAGCGTGAC	CCCGCCGCTG	GACTTTTGGT	AGGACGTATG
15101	CAACATGCCA	AATGTGAACG	AGTTCATGTT	TACCAATAAG	TTTAAGGCGC
	GTTGTACGGT	TTACACTTGC	TCAAGTACAA	ATGGTTATTC	AAATTCCGCG

Figure 26 P

15151	GGGTGATGGT CCCACTACCA	CAGCGCGAAC	CCTACTAAGG GGATGATTCC	ACAATCAGGT TGTTAGTCCA	GGAGCT LA CCTCGACTTT
15201				GGCAACTACT CCGTTGATGA	
15251				GGAGCACTAC CCTCGTGATG	
15301				TCGGGGTAAA AGCCCCATTT	
15351	CGCAACTTCA	GACTGGGGTT	TGACCCCGTC	ACTGGTCTTG	TCATGCCTGG
	GCGTTGAAGT	CTGACCCCAA	ACTGGGGCAG	TGACCAGAAC	AGTACGGACC
15401				CATCATTTTG GTAGTAAAAC	
15451				GCAACTTGTT CGTTGAACAA	
15501	AAGCGGCAAC	CCTTCCAGGA	GGGCTTTAGG	ATCACCTACG	ATGATCTGGA
	TTCGCCGTTG	GGAAGGTCCT	CCCGAAATCC	TAGTGGATGC	TACTAGACCT
15551				GGAÇGCCTAC CCTGCGGATG	
15601	TGAAAGATGA ACTTTCTACT	CACCGAACAG GTGGCTTGTC	GGCGGGGGTG	GCGCAGGCGG CGCGTCCGCC	CAGCAACAGC GTCGTTGTCG
15651	AGTGGCAGCG TCACCGTCGC	GCGCGGAAGA CGCGCCTTCT	GAACTCCAAC CTTGAGGTTG	GCGGCAGCCG	CGGCAATGCA GCCGTTACGT
15701	GCCGGTGGAG	GACATGAACG	ATCATGCCAT	TCGCGGCGAC	ACCTTTGCCA
	CGGCCACCTC	CTGTACTTGC	TAGTACGGTA	AGCGCCGCTG	TGGAAACGGT
15751	CACGGGCTGA	GGAGAAGCGC	GCTGAGGCCG	AAGCAGCGGC	CGAAGCTGCC
	GTGCCCGACT	CCTCTTCGCG	CGACTCCGGC	TTCGTCGCCG	GCTTCGACGG
15801	GCCCCCGCTG	CGCAACCCGA	GGTCGAGAAG	CCTCAGAAGA	AACCGGTGAT
	CGGGGGCGAC	GCGTTGGGCT	CCAGCTCTTC	GGAGTCTTCT	TTGGCCACTA
15851	CAAACCCCTG	ACAGAGGACA	GCAAGAAACG	CAGTTACAAC	CTAATAAGCA
	GTTTGGGGAC	TGTCTCCTGT	CGTTCTTTGC	GTCAATGTTG	GATTATTCGT
15901	ATGACAGCAC	CTTCACCCAG	TACCGCAGCT	GCTACCTTGC	ATACAACTAC
	TACTGTCGTG	GAAGTGGGTC	ATGGCGTCGA	CCATGGAACG	TATGTTGATG
15951	GGCGACCCTC	AGACCGGAAT	CCGCTCATGG	ACCCTGCTTT	GCACTCCTGA
	CCGCTGGGAG	TCTGGCCTTA	GGCGAGTACC	TGGGACGAAA	CGTGAGGACT
160,01	CGTAACCTGC	GGCTCGGAGC	AGGTCTACTG	GTCGTTGCCA	GACATGATGC
	GCATTGGACG	CCGAGCCTCG	TCCAGATGAC	CAGCAACGGT	CTGTACTACG
16051	AAGACCCCGT	GACCTTCCGC	TCCACGCGCC	AGATCAGCAA	CTTTCCGGTG
	TTCTGGGGCA	CTGGAAGGCG	AGGTGCGCGG	TCTAGTCGTT	GAAAGGCCAC

Figure 26 Q

16101	GTGGGCGCCG	A TGTTGCC	CGTGCACTCC	AAGAGCTTCT	ACAACGA CA
	CACCCGCGGC	TLACAACGG	GCACGTGAGG	TTCTCGAAGA	TGTTGC GT
16151	GGCCGTCTAC	TCCCAACTCA	TCCGCCAGTT	TACCTCTCTG	ACCCACGTGT
	CCGGCAGATG	AGGGTTGAGT	AGGCGGTCAA	ATGGAGAGAC	TGGGTGCACA
16201	TCAATCGCTT	TCCCGAGAAC	CAGATTTTGG	GCGCGGGCGG	AGCCCCCACC
	AGTTAGCGAA	AGGGCTCTTG	GTCTAAAACC	CGCGCCCGCC	TCGGGGGTGG
16251	ATCACCACCG	TCAGTGAAAA	CGTTCCTGCT	CTCACAGATC	ACGGGACGCT
	TAGTGGTGGC	AGTCACTTTT	GCAAGGACGA	GAGTGTCTAG	TGCCCTGCGA
16301	ACCGCTGCGC	AACAGCATCG	GAGGAGTCCA	GCGAGTGACC	ATTACTGACG
	TGGCGACGCG	TTGTCGTAGC	CTCCTCAGGT	CGCTCACTGG	TAATGACTGC
16351	CCAGACGCCG	CACCTGCCCC	TACGTTTACA	AGGCCCTGGG	CATAGTCTCG
	GGTCTGCGGC	GTGGACGGGG	ATGCAAATGT	TCCGGGACCC	GTATCAGAGC
16401	CCGCGCGTCC	TATCGAGCCG	CACTTTTTGA	GCAAGCATGT	CCATCCTTAT
	GGCGCGCAGG	ATAGCTCGGC	GTGAAAAACT	CGTTCGTACA	GGTAGGAATA
16451	ATCGCCCAGC	AATAACACAG	GCTGGGGCCT	GCGCTTCCCA	AGCAAGATGT
	TAGCGGGTCG	TTATTGTGTC	CGACCCCGGA	CGCGAAGGGT	TCGTTCTACA
16501	TTGGCGGGGC AACCGCCCCG	CAAGAAGCGC GTTCTTCGCG	TCCGACCAAC AGGCTGGTTG	ACCCAGTGCG TGGGTCACGC	CCTGCGCGGG
16551	CACTACCGCG GTGATGGCGC	CGCCCTGGGG GCGGGACCCC	CGCGCACAAA GCGCGTGTTT	CGCGGCCGCGT	CTGGGCGCAC GACCCGCGTG
16601	CACCGTCGAT	GACGCCATCG	ACGCGGTGGT	GGAGGAGGCG	CGCAACTACA
	GTGGCAGCTA	CTGCGGTAGC	TGCGCCACCA	CCTCCTCCGC	GCGTTGATGT
16651	CGCCCACGCC	GCCACCAGTG CGGTGGTCAC	TCCACAGTGG AGGTGTCACC	ACGCGGCCAT TGCGCCGGTA	TCAGACCGTG AGTCTGGCAC
16701	GTGCGCGGAG	CCCGGCGCTA	TGCTAAAATG	AAGAGACGGC	GGAGGCGCGT
	CACGCGCCTC	GGGCCGCGAT	ACGATTTTAC	TTCTCTGCCG	CCTCCGCGCA
16751	AGCACGTCGC TCGTGCAGCG	CACCGCCGCC GTGGCGGCGG	GACCCGGCAC CTGGGCCGTG	TGCCGCCCAA ACGGCGGGTT	00000000000000000000000000000000000000
16801	CGGCCCTGCT GCCGGGACGA	TAACCGCGCA ATTGGCGCGT	CGTCGCACCG GCAGCGTGGC	GCCGACGGGC	GGCCATGCGG CCGGTACGCC
16851	GCCGCTCGAA CGGCGAGCTT	GGCTGGCCGC	GGGTATTGTC CCCATAACAG	ACTGTGCCCC TGACACGGGG	CCAGGTCCAG GGTCCAGGTC
16901	GCGACGAGCG	GCCGCCGCAG	CAGCCGCGCGC	CATTAGTGCT	ATGACTCAGG
	CGCTGCTCGC	CGGCGGCGTC	GTCGGCGCCCG	GTAATCACGA	TACTGAGTCC
16951	GTCGCAGGGG CAGCGTCCCC	CAACGTGTAT GTTGCACATA	TGGGTGCGCG	ACTCGGTTAG TGAGCCAATC	CGGCCTGCGC
17001	GTGCCCGTGC CACGGGCACG	GCACCCGCCC CGTGGGCGGG	CCCGCGCAAC	TAGATTGCAA ATCTAACGTT	GAAAAAACTA CTTTTTTGAT



17051	CTTAGACTCG GAATCTGAGC	T GTTGTA ATGACAACAT	TGTATCCAGC ACATAGGTCG	CCCCCCCCCC	GCGTTGCTTC
17101			AAAGAAGAGA TTTCTTCTCT		
17151			GAAGGAAGAG CTTCCTTCTC		
17201			AAAAGAAAGA TTTTCTTTCT		
17251			GCTACCGCGC CGATGGCGCG		
17301			TGTTTTGCGA ACAAAACGCT		
17351			CCCGCACCTA GGGCGTGGAT		
17401			CTTGAGCAGG GAACTCGTCC		
17451			TAAGGACATG ATTCCTGTAC		
17501			TAAAGCCCGT ATTTCGGGCA		
17551			GAAAAGCGCG CTTTTCGCGC		
17601			GCTGATGGTA CGACTACCAT		
17651			CCGTGGAACC GGCACCTTGG		
17701			GTGGCGCCGG CACCGCGGCC		
17751	GACGTTCAGA CTGCAAGTCT	TACCCACTAC ATGGGTGATG	CAGTAGCACC GTCATCGTGG	AGTATTGCCA TCATAACGGT	CCGCCACAGA GGCGGTGTCT
17801	GGGCATGGAG CCCGTACCTC	ACACAAACGT TGTGTTTGCA	CCCCGGTTGC GGGGCCAACG	CTCAGCGGTG GAGTCGCCAC	GCGGATGCCG
17851	CGGTGCAGGC GCCACGTCCG	GGTCGCTGCG CCAGCGACGC	GCCGCGTCCA CGGCGCAGGT	AGACCTCTAC TCTGGAGATG	GGAGGTGCAA CCTCCACGTT
17901	ACGGACCCGT TGCCTGGGCA	GGATGTTTCG CCTACAAAGC	CGTTTCAGCC GCAAAGTCGG	CCCCGGCGCC	CGCGCCGTTC
17951	GAGGAAGTAC CTCCTTCATG	GGCGCCGCCA CCGCGGCGGT	GCGCGCTACT CGCGCGATGA	GCCCGAATAT CGGGCTTATA	GCCCTACATC CGGGATGTAG

Figure 265

18001	CTTCCATTGC GAAGGTAACG	GCCTACCCC CTATGGGGG	GGCTATCGTG CCGATAGCAC	GCTACACCTAL CGATGTGGAT	GGCGGGGT T
18051	AGACGAGCAA TCTGCTCGTT	CTACCCGACG GATGGGCTGC	CCGAACCACC GGCTTGGTGG	ACTGGAACCC TGACCTTGGG	CGCCGCCGC
18101	TCGCCGTCGC	CAGCCCGTGC	TGGCCCCGAT	TTCCGTGCGC	AGGGTGGCTC
	AGCGGCAGCG	GTCGGGCACG	ACCGGGGCTA	AAGGCACGCG	TCCCACCGAG
18151	GCGAAGGAGG	CAGGACCCTG	GTGCTGCCAA	CAGCGCGCTA	CCACCCCAGC
	CGCTTCCTCC	GTCCTGGGAC	CACGACGGTT	GTCGCGCGAT	GGTGGGGTCG
18201	ATCGTTTAAA	AGCCGGTCTT	TGTGGTTCTT	GCAGATATGG	CCCTCACCTG
	TAGCAAATTT	TCGGCCAGAA	ACACCAAGAA	CGTCTATACC	GGGAGTGGAC
18251	CCGCCTCCGT	TTCCCGGTGC	CGGGATTCCG	AGGAAGAATG	CACCGTAGGA
	GGCGGAGGCA	AAGGGCCACG	GCCCTAAGGC	TCCTTCTTAC	GTGGCATCCT
18301	GGGGCATGGC	CGGCCACGGC	CTGACGGGCG	GCATGCGTCG	TGCGCACCAC
	CCCCGTACCG	GCCGGTGCCG	GACTGCCCGC	CGTACGCAGC	ACGCGTGGTG
18351	CGCCGCCGC	GCGCGTCGCA CGCGCAGCGT	CCGTCGCATG GGCAGCGTAC	CGCGGCGGTA GCGCCGCCAT	TCCTGCCCT AGGACGGGA
18401	CCTTATTCCA	CTGATCGCCG	CGGCGATTGG	CGCCGTGCCC	GGAATTGCAT
	GGAATAAGGT	GACTAGCGGC	GCCGCTAACC	GCGGCACGGG	CCTTAACGTA
18451	CCGTGGCCTT	GCAGGCGCAG	AGACACTGAT	TAAAAACAAG	TTGCATGTGG
	GGCACCGGAA	CGTCCGCGTC	TCTGTGACTA	ATTTTTGTTC	AACGTACACC
18501	AAAAATCAAA	ATAAAAAGTC	TGGACTCTCA	CGCTCGCTTG	GTCCTGTAAC
	TTTTTAGTTT	TATTTTTCAG	ACCTGAGAGT	GCGAGCGAAC	CAGGACATTG
18551	TATTTTGTAG	AATGGAAGAC	ATCAACTTTG	CGTCTCTGGC	CCCGCGACAC
	ATAAAACATC	TTACCTTCTG	TAGTTGAAAC	GCAGAGACCG	GGGCGCTGTG
18601	GGCTCGCGCC	CGTTCATGGG	AAACTGGCAA	GATATCGGCA	CCAGCAATAT
	CCGAGCGCGG	GCAAGTACCC	TTTGACCGTT	CTATAGCCGT	GGTCGTTATA
18651	GAGCGGTGGC	GCCTTCAGCT	GGGGCTCGCT	GTGGAGCGGC	TTAAAAATT
	CTCGCCACCG	CGGAAGTCGA	CCCCGAGCGA	CACCTCGCCG	AATTTTTAA
18701	TCGGTTCCAC	CGTTAAGAAC	TATGGCAGGA	AGGCCTGGAA	CAGCAGCACA
	AGCCAAGGTG	GCAATTCTTG	ATACCGTCGT	TCCGGACCTT	GTCGTCGTGT
18751	GGCCAGATGC	TGAGGGATAA	GTTGAAAGAG	CAAAATTTCC	AACAAAAGGT
	CCGGTCTACG	ACTCCCTATT	CAACTTTCTC	GTTTTAAAGG	TTGTTTTCCA
18801	GGTAGATGGC	CTGGCCTCTG	GCATTAGCGG	GGTGGTGGAC	CTGGCCAACC
	CCATCTACCG	GACCGGAGAC	CGTAATCGCC	CCACCACCTG	GACCGGTTGG
18851	AGGCAGTGCA	AAATAAGATT	AACAGTAAGC	TTGATCCCCG	CCCTCCCGTA
	TCCGTCACGT	TTTATTCTAA	TTGTCATTCG	AACTAGGGGC	GGGAGGGCAT
18901	GAGGAGCCTC	CACCGGCCGT	GGAGACAGTG	TCTCCAGAGG	GCCGTGGCGA
	CTCCTCGGAG	GTGGCCGGCA	CCTCTGTCAC	AGAGGTCTCC	CCGCACCGCT

Figure 26T

18951	AAAGCGTCCG TTTCGCAGGC	CCGACA GCGGGCTGT	GGGAAGAAAC CCCTTCTTTG	TCTGGTGACG AGACCACTGC	CAAATA G
19001		GTACGAGGAG CATGCTCCTC			
19051		CCATGGCTAC GGTACCGATG			
19101		CCTCCCCCG			
19151		CGTTGTTGTA GCAACAACAT			
19201		GTCCGCGATC CAGGCGCTAG			
19251		AACAGCATCG TTGTCGTAGC			
19301		CTGATAGCTA GACTATCGAT			
19351		CAGAGGAGCT GTCTCCTCGA			
19401		TTCGATGATG AAGCTACTAC			
19451		CGGAGTACCT GCCTCATGGA			
19501		TACTTCAGCC ATGAAGTCGG			
19551		CGACGTGACC GCTGCACTGG			
19601		TGGACCGTGA ACCTGGCACT			
19651		GTGGGTGATA CACCCACTAT			
19701	TTGACATCCG AACTGTAGGC	CGGCGTGCTG GCCGCACGAC	GACAGGGGCC CTGTCCCCGG	CTACTTTTAA GATGAAAATT	GCCCTACTCT CGGGATGAGA
19751	GGCACTGCCT CCGTGACGGA	ACAACGCCCT TGTTGCGGGA			
19801	ATGGGATGAA TACCCTACTT	GCTGCTACTG CGACGATGAC	CTCTTGAAAT GAGAACTTTA	AAACCTAGAA TTTGGATCTT	GAAGAGGACG CTTCTCCTGC
19851	ATGACAACGA TACTGTTGCT	AGACGAAGTA TCTGCTTCAT			

Figure 26 U

19901 .	GTATTTGGGC CATAAACCCG	A GCCTTA TCCGCGGAAT	TTCTGGTATA AAGACCATAT	AATATTACAA TTATAATGTT	AGGAGG TA
19951		CAGCTTCCAG	TTTGTGGATT	TATACGGCTA	TTTTGTAAAG
20001		AGTTTATCCT	CTTAGAGTCA	CCATGCTTTG	TCTTTAATTA
20051	GTACGTCGAC	CCTCTCAGGA	TTTTTTCTGA	ACCCCAATGA TGGGGTTACT	TTGGTACAAT
20101	GCCAAGTATA	CGTTTTGGGT	GTTTACTTTT	TGGAGGGCAA ACCTCCCGTT	CCGTAAGAAC
20151	ATTTCGTTGT	TTTACCTTTC	GATCTTTCAG	AAGTGGAAAT TTCACCTTTA	CGTTAAAAAG
20201	AGTTGATGAC	TCCGTCGGCG	TCCGTTACCA	GATAACTTGA CTATTGAACT	GAGGATTTCA
20251	CCATAACATG	TCACTTCTAC	ATCTATATCT		TGAGTATAAA
20301	GAATGTACGG	GTGATAATTC	CTTCCATTGA	CACGAGAACT GTGCTCTTGA	TTACCCGGTT
20351	GTTAGATACG	GGTTGTCCGG	ATTAATGTAA	GCTTTTAGGG CGAAAATCCC	TGITAAAATA
20401	ACCAGATTAC	ATAATGTTGT	CGTGCCCATT	TATGGGTGTT ATACCCACAA	GACCGCCCGG
20451	TTCGTAGCGT	CAACTTACGA	CAACATCTAA	TGCAAGACAG ACGTTCTGTC	TTTGTGTCTC
20501	GAAAGTATGG	TCGAAAACGA	ACTAAGGTAA	GGTGATAGAA CCACTATCTT	GGTCCATGAA
20551	AAGATACACC	TTAGTCCGAC	AACTGTCGAT	TGATCCAGAT ACTAGGTCTA	CAATCTTAAT
20601	AACTTTTAGT	ACCTTGACTT	CTACTTGAAG	CAAATTACTG GTTTAATGAC	GAAAGGTGAC
		AATTATGTCT	CTGAGAATGG	TTCCATTTTG	GATTTTGTCC
	AGTCCTTTTA	CCTACCCTTT	TTCTACGATG	TCTTAAAAGT	GATAAAAATG CTATTTTTAC
	TTTATTCTCA	ACCTTTATTA	AAACGGTACC	TTTAGTTAGA	AAATGCCAAC TTTACGGTTG
20801	CTGTGGAGAA GACACCTCTT	ATTTCCTGTA TAAAGGACAT	CTCCAACATA GAGGTTGTAT	GCGCTGTATT CGCGACATAA	TGCCCGACAA ACGGGCTGTT

Tigure 26 V

20851	GCTAAAGTAC CGATTTCATG	ACCTTCCA TGGAAGGT	ACGTAAAAAT TGCATTTTTA	TTCTGATÄÄČ AAGACTATTG	T SAKADT A STTTDD
20901	ACGACTACAT TGCTGATGTA	GAACAAGCGA CTTGTTCGCT	GTGGTGGCTC CACCACCGAG	CCGGGCTAGT GGCCCGATCA	GGACTGCTAC CCTGACGATG
20951				TATATGGACA ATATACCTGT	
21001	TAAATTGGTG	GTGGCGTTAC	GACCGGACGC	CTACCGCTCA GATGGCGAGT	TACAACGACC
21051	CGTTACCAGC	GATACACGGG	AAGGTGTAGG	AGGTGCCTCA TCCACGGAGT	CTTCAAGAAA
21101	CGGTAATTTT	TGGAGGAAGA	GGACGGCCCG	TCATACACCT AGTATGTGGA	TGCTCACCTT
21151	GAAGTCCTTC	CTACAATTGT	ACCAAGACGT	GAGCTCCCTA CTCGAGGGAT	CCTTTACTGG
21201	ATTCCCAACT	GCCTCGGTCG	TAATTCAAAC	ATAGCATTTG TATCGTAAAC	GGAAATGCGG
21251	TGGAAGAAGG	GGTACCGGGT	GTTGTGGCGG	TCCACGCTTG AGGTGCGAAC	TCCGGTACGA
21301	ATCTTTGCTG	TGGTTGCTGG	TCAGGAAATT	CGACTATCTC GCTGATAGAG	AGGCGGCGGT
21351	TGTACGAGAT	GGGATATGGG	CGGTTGCGAT	CCAACGTGCC GGTTGCACGG	GTATAGGTAG
21401	GGGAGGGCGT	TGACCCGCCG	AAAGGCGCCG	TGGGCCTTCA ACCCGGAAGT	GCGCGGAATT
21451	CTGATTCCTT	TGGGGTAGTG	ACCCGAGCCC	CTACGACCCT GATGCTGGGA	ATAATGTGGA
21501	TGAGACCGAG	ATATGGGATG	GATCTACCTT	CCTTTTACCT GGAAAATGGA	GTTGGTGTGG
21551	AAATTCTTCC	ACCGGTAATG	GAAACTGAGA	TCTGTCAGCT AGACAGTCGA	CCGGACCGTT
		GAATGGGGGT	TGCTCAAACT	TTAATTCGCG	AGTCAACTGC
		GTTGCAACGG	GTCACATTGT	ACTGGTTTCT	GACCAAGGAC
		ATCGATTGAT	ATTGTAACCG	ATGGTCCCGA	AGATATAGGG
21751	AGAGAGCTAC TCTCTCGATG	AAGGACCGCA TTCCTGGCGT	TGTACTCCTT ACATGAGGAA	CTTTAGAAAC GAAATCTTTG	TTCCAGCCCA AAGGTCGGGT

Figure 26 W

21801	TGAGCCGTCA	GOTGGTGGAT	GATACÎAAAT CTATGATTTA	ACAAGGACTAL TGTTCCTGAT	CCAACAGETG GGTTG1
21851		_		TTTGTTGGCT	
	CCGTAGGATG	TGGTTGTGTT	GTTGAGACCT	AAACAACCGA	TGGAACGGGG
21901	CACCATGCGC GTGGTACGCG	GAAGGACAGG CTTCCTGTCC	CCTACCCTGC GGATGGGACG	TAACTTCCCC ATTGAAGGGG	TATCCGCTTA ATAGGCGAAT
21951	TAGGCAAGAC	CGCAGTTGAC	AGCATTACCC	AGAAAAAGTT	TCTTTGCGAT
				TCTTTTTCAA AACTTTATGT	
22001	GCGTGGGAAA	CCGCGTAGGG	TAAGAGGTCA	TTGAAATACA	GGTACCCGCG
22051	ACTCACAGAC TGAGTGTCTG	CTGGGCCAAA GACCCGGTTT	ACCTTCTCTA TGGAAGAGAT	CGCCAACTCC GCGGTTGAGG	GCCCACGCGC CGGGTGCGCG
22101	· TAGACATGAC	TTTTGAGGTG	GATCCCATGG	ACGAGCCCAC	CCTTCTTTAT
				TGCTCGGGTG	
22151	GTTTTGTTTG CAAAACAAAC	AAGTCTTTGA TTCAGAAACT	GCACCAGGCA	GTGCACCAGC CACGTGGTCG	GCGTGGCGCC
22201	CGTCATCGAA GCAGTAGCTT	ACCGTGTACC TGGCACATGG	TGCGCACGCC ACGCGTGCGG	CTTCTCGGCC GAAGAGCCGG	GGCAACGCCA CCGTTGCGGT
22251	CAACATAAAG	AAGCAAGCAA	CATCAACAAC	AGCTGCCGCC	ATGGGCTCCA
				TCGACGGCGG	
22301	GTGAGCAGGA CACTCGTCCT	ACTGAAAGCC TGACTTTCGG	ATTGTCAAAG TAACAGTTTC	ATCTTGGTTG TAGAACCAAC	ACCCGGTATA
22351	TTTTTGGGCA	CCTATGACAA	GCGCTTTCCA CGCGAAAGGT	GGCTTTGTTT CCGAAACAAA	CTCCACACAA GAGGTGTGTT
22401	GCTCGCCTGC	GCCATAGTCA	ATACGGCCGG	TCGCGAGACT	GGGGGCGTAC
	CGAGCGGACG	CGGTATCAGT	TATGCCGGCC	AGCGCTCTGA	CCCCCGCATG
22451	ACTGGATGGC TGACCTACCG	CTTTGCCTGG GAAACGGACC	AACCCGCACT TTGGGCGTGA	CAAAAACATG GTTTTTGTAC	GATGGAGAAA
22501	GAGCCCTTTG CTCGGGAAAC	GCTTTTCTGA CGAAAAGACT	CCAGCGACTC GGTCGCTGAG	AAGCAGGTTT TTCGTCCAAA	ACCAGTTTGA TGGTCAAACT
22551	GTACGAGTCA CATGCTCAGT	CTCCTGCGCC GAGGACGCGG	GTAGCGCCAT CATCGCGGTA	TGCTTCTTCC ACGAAGAAGG	CCCGACCGCT GGGCTGGCGA
22601	GTATAACGCT CATATTGCGA	GGAAAAGTCC CCTTTTCAGG	ACCCAAAGCG TGGGTTTCGC	TACAGGGGCC ATGTCCCCGG	CAACTCGGCC GTTGAGCCGG
22651	GCCTGTGGAC CGGACACCTG	TATTCTGCTG ATAAGACGAC	CATGTTTCTC GTACAAAGAG	CACGCCTTTG GTGCGGAAAC	CCAACTGGCC GGTTGACCGG
22701	CCAAACTCCC GGTTTGAGGG	ATGGATCACA TACCTAGTGT	ACCCCACCAT TGGGGTGGTA	GAACCTTATT CTTGGAATAA	ACCGGGGTAC TGGCCCCATG

Figure 26 X

22751	CCAACTCCAT GGTTGAGGTA	GCTCAACAGT CTTGTCA	CCCCAGGTAC GGGGTCCATG	AGCCCACQGA TCGGGTGGGA	ccyccowyc
22801			CCTGGAGCGC GGACCTCGCG		
22851	CCACAGTGCG GGTGTCACGC	CAGATTAGGA GTCTAATCCT	GCGCCACTTC CGCGGTGAAG	TTTTTGTCAC AAAAACAGTG	TTGAAAAACA AACTTTTTGT
22901			GACACTTTCA CTGTGAAAGT		
22951			ATTTACCCCC TAAATGGGGG		
23001			GCCGCGCATC CGGCGCGTAG		
23051			TTAGTGCTCC AATCACGAGG		
23101			GTTTTCACTC CAAAAGTGAG		
23151			GCGCCGATAT CGCGGCTATA		
23201			TTGCGATACA AACGCTATGT		
23251			CACGCTGGCC GTGCGACCGG		
23301			CCGCGTTGCT GGCGCAACGA		
23351			AAGGGCGCGT TTCCCGCGCA	•	
23401			AAGGTGACCG TTCCACTGGC		
23451			CCTTGATCTG GGAACTAGAC		
	TTGCGCCTTC AACGCGGAAG				
23551	GCCGGACAGG CGGCCTGTCC		CACGCAGCAC GTGCGTCGTG		
23601	CTGCACCACA GACGTGGTGT		ACCGGTTCTT TGGCCAAGAA		
23651	ACTGCTCCTT TGACGAGGAA		TGCCCGTTTT ACGGGCAAAA		

Figure 26 Y

23701	ATCACGTGCT TAGTGCACGA	CCTATTTAT GGAATAAATA	CATAATGCTT GTATTACGAA	CCGTGTAGAC GGCACATCTG	ACTTAA CC TGAATTCGAG
23751	GCCTTCGATC CGGAAGCTAG	TCAGCGCAGC AGTCGCGTCG	GGTGCAGCCA CCACGTCGGT	CAACGCGCAG GTTGCGCGTC	CCCGTGGGCT GGGCACCCGA
23801	CGTGATGCTT GCACTACGAA	GTAGGTCACC CATCCAGTGG	TCTGCAAACG AGACGTTTGC	ACTGCAGGTA TGACGTCCAT	CGCCTGCAGG GCGGACGTCC
23851	AATCGCCCCA TTAGCGGGGT	TCATCGTCAC AGTAGCAGTG	AAAGGTCTTG TTTCCAGAAC	TTGCTGGTGA AACGACCACT	AGGTCAGCTG TCCAGTCGAC
23901	CAACCCGCGG GTTGGGCGCC	TGCTCCTCGT ACGAGGAGCA	TCAGCCAGGT AGTCGGTCCA	CTTGCATACG GAACGTATGC	GCCGCCAGAG CGGCGGTCTC
23951	CTTCCACTTG GAAGGTGAAC	GTCAGGCAGT CAGTCCGTCA	AGTTTGAAGT TCAAACTTCA	TCGCCTTTAG AGCGGAAATC	ATCGTTATCC TAGCAATAGG
24001	TGCACCATGA	ACAGGTAGTC	CGCGCGCGCA GCGCGCGCGT	CGGAGGTACG	GGAAGAGGGT
24051	GCGTCTGTGC	TAGCCGTGTG	TCAGCGGGTT AGTCGCCCAA	GTAGTGGCAT	TAAAGTGAAA
24101	GGCGAAGCGA	CCCGAGAAGG	TCTTCCTCTT AGAAGGAGAA	CGCAGGCGTA	TGGTGCGCGG
24151	TGACCCAGCA	GAAGTAAGTC	CCGCCGCACT GGCGGCGTGA	CACGCGAATG	GAGGAAACGG
24201	TACGAACTAA	TCGTGGCCAC	CCAACGACTT	TGGGTGGTAA	
24251	GTAGAAGAGA	AAGAAGGAGC	CTGTCCACGA GACAGGTGCT	AATGGAGACC	ACTACCGCCC
24301	GCGAGCCCGA	ACCCTCTTCC	GCGCTTCTTT CGCGAAGAAA	AAGAAGAACC	CGCGTTACCG
24351	GTTTAGGCGG	CGGCTCCAGC	TACCGGCGCC	CGACCCACAC	CGCGGCACCA GCGCCGTGGT
24401	CGCGCAGAAC	ACTACTCAGA	AGGAGCAGGA	GCCTGAGCTA	ACGCCGCCTC TGCGGCGGAG
	TAGGCGAAAA	AACCCCCGCG	GGCCCCTCCG	CCGCCGCTGC	GGGACGGGGA CCCTGCCCCT
	GCTGTGCAGG	AGGTACCAAC	CCCCTGCAGC	GCGGCGTGGC	CGTCCGCGCT GCAGGCGCGA
	GCCCCCACCA	AAGCGCGACG	AGGAGAAGGG	CTGACCGGTA	TTCCTTCTCC AAGGAAGAGG
24601	TATAGGCAGA ATATCCGTCT	AAAAGATCAT TTTTCTAGTA	GGAGTCAGTC CCTCAGTCAG	GAGAAGAAGG CTCTTCTTCC	ACAGCCTAAC TGTCGGATTG

Figure 262

24651	CGCCCCCTCT GCGGGGGAGA	TCGCCA CTCAAGCGGT	CCACCGCCTC GGTGGCGGAG	CACCGATGCC GTGGCTACGG	GCCAAC CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC
24701				TTGAGGAGGA AACTCCTCCT	
24751				GACGACGAGG CTGCTGCTCC	
24801				CAACGCAGAG GTTGCGTCTC	
24851				GCGACTACCT CGCTGATGGA	
24901				CAGTGCGCCA GTCACGCGGT	
24951				CGCCATAGCG GCGGTATCGC	
25001				GCGTACCCCC CGCATGGGGG	
25051				CTCAACTTCT GAGTTGAAGA	
25101				CATCTTTTTC GTAGAAAAAG	
25151				GCCGAGCGGA CGGCTCGCCT	
25201				ATCGCCTCGC TAGCGGAGCG	
25251				CGAGAAGCGC GCTCTTCGCG	
25301	0.0.00			GTCACTCTGG CAGTGAGACC	
25351				GTACTAAAAC CATGATTTTG	
25401	GGTCACCCAC CCAGTGGGTG			CCTACCCCC GGATGGGGGG	
25451	GCACAGTCAT CGTGTCAGTA			GTGCGCAGCC CACGCGTCGG	
25501	GATGCAAATT CTACGTTTAA			GGCCTACCCG CCGGATGGGC	
25551	CCAGCAGCTA GCTCGTCGAT			CGAGCCTGCC GCTCGGACGG	

7 igure 26 AA

25601	AGCGACGCAA	AATGATG	GCCGCAGTGC	TCGTTACCGT	GGAGCT G
	TCGCTGCGTT	TGATTACTAC	CGGCGTCACG	AGCAATGGCA	CCTCGAACTC
25651	TGCATGCAGC	GGTTCTTTGC	TGACCCGGAG	ATGCAGCGCA	AGCTAGAGGA
	ACGTACGTCG	CCAAGAAACG	ACTGGGCCTC	TACGTCGCGT	TCGATCTCCT
25701	AACATTGCAC	TACACCTTTC	GACAGGGCTA	CGTACGCCAG	GCCTGCAAGA
	TTGTAACGTG	ATGTGGAAAG	CTGTCCCGAT	GCATGCGGTC	CGGACGTTCT
25751	TCTCCAACGT	GGAGCTCTGC	AACCTGGTCT	CCTACCTTGG	AATTTTGCAC
	AGAGGTTGCA	CCTCGAGACG	TTGGACCAGA	GGATGGAACC	TTAAAACGTG
25801	GAAAACCGCC	TTGGGCAAAA	CGTGCTTCAT	TCCACGCTCA	AGGGCGAGGC
	CTTTTGGCGG	AACCCGTTTT	GCACGAAGTA	AGGTGCGAGT	TCCCGCTCCG
25851	GCGCCGCGAC	TACGTCCGCG	ACTGCGTTTA	CTTATTTCTA	TGCTACACCT
	CGCGGCGCTG	ATGCAGGCGC	TGACGCAAAT	GAATAAAGAT	ACGATGTGGA
25901	GGCAGACGGC	CATGGGCGTT	TGGCAGCAGT	GCTTGGAGGA	GTGCAACCTC
	CCGTCTGCCG	GTACCCGCAA	ACCGTCGTCA	CGAACCTCCT	CACGTTGGAG
25951	AAGGAGCTGC	AGAAACTGCT	AAAGCAAAAC	TTGAAGGACC	TATGGACGGC
	TTCCTCGACG	TCTTTGACGA	TTTCGTTTTG	AACTTCCTGG	ATACCTGCCG
26001	CTTCAACGAG	CGCTCCGTGG	CCGCGCACCT	GGCGGACATC	ATTTTCCCCG
	GAAGTTGCTC	GCGAGGCACC	GGCGCGTGGA	CCGCCTGTAG	TAAAAGGGGC
26051	AACGCCTGCT	TAAAACCCTG	CAACAGGGTC	TGCCAGACTT	CACCAGTCAA
	TTGCGGACGA	ATTTTGGGAC	GTTGTCCCAG	ACGGTCTGAA	GTGGTCAGTT
26101	AGCATGTTGC	AGAACTTTAG	GAACTTTATC	CTAGAGCGCT	CAGGAATCTT
	TCGTACAACG	TCTTGAAATC	CTTGAAATAG	GATCTCGCGA	GTCCTTAGAA
26151	GCCCGCCACC	TGCTGTGCAC	TTCCTAGCGA	CTTTGTGCCC	ATTAAGTACC
	CGGGCGGTGG	ACGACACGTG	AAGGATCGCT	GAAACACGGG	TAATTCATGG
26201	GCGAATGCCC	TCCGCCGCTT	TGGGGCCACT	GCTACCTTCT	GCAGCTAGCC
	CGCTTACGGG	AGGCGGCGAA	ACCCCGGTGA	CGATGGAAGA	CGTCGATCGG
26251	AACTACCTTG	CCTACCACTC	TGACATAATG	GAAGACGTGA	GCGGTGACGG
	TTGATGGAAC	GGATGGTGAG	ACTGTATTAC	CTTCTGCACT	CGCCACTGCC
26301	TCTACTGGAG	TGTCACTGTC	GCTGCAACCT	ATGCACCCCG	CACCGCTCCC
	AGATGACCTC	ACAGTGACAG	CGACGTTGGA	TACGTGGGGC	GTGGCGAGGG
26351	TGGTTTGCAA ACCAAACGTT	TTCGCAGCTG AAGCGTCGAC	CTTAACGAAA GAATTGCTTT	GTCAAATTAT CAGTTTAATA	CGGTACCTTT
26401	GAGCTGCAGG	GTCCCTCGCC	TGACGAAAAG	TCCGCGGCTC	CGGGGTTGAA
	CTCGACGTCC	CAGGGAGCGG	ACTGCTTTTC	AGGCGCCGAG	GCCCCAACTT
26451	ACTCACTCCG	GGGCTGTGGA	CGTCGGCTTA	CCTTCGCAAA	TTTGTACCTG
	TGAGTGAGGC	CCCGACACCT	GCAGCCGAAT	GGAAGCGTTT	AAACATGGAC
26501	AGGACTACCA	CGCCCACGAG	ATTAGGTTCT	ACGAAGACCA	ATCCCGCCCG
	TCCTGATGGT	GCGGGTGCTC	TAATCCAAGA	TGCTTCTGGT	TAGGGCGGGC

Figure 26 AB

26551				ACCCAGGGCC TGGGTCCCGG	
26601				AGAGTTTCTG TCTCAAAGAC	
26651	GACGGGGGGT CTGCCCCCCA	TTACTTGGAC AATGAACCTG	CCCCAGTCCG GGGGTCAGGC	GCGAGGAGCT CGCTCCTCGA	CAACCCAATC GTTGGGTTAG
26701				GCGCGGGCCC	
26751	GGATGGCACC CCTACCGTGG	CAAAAAGAAG GTTTTTCTTC	CTGCAGCTGC GACGTCGACG	CGCCGCCACC GCGGCGGTGG	CACGGACGAG GTGCCTGCTC
26801	GAGGAATACT CTCCTTATGA	GGGACAGTCA CCCTGTCAGT	GGCAGAGGAG CCGTCTCCTC	GTTTTGGACG CAAAACCTGC	AGGAGGAGGA TCCTCCTCCT
26851				CGAGGAAGCT GCTCCTTCGA	
26901	TTCTCCACAG	TCTGCTTTGT	GGCAGTGGGA	CGGTCGCATT GCCAGCGTAA	GGGGAGCGGC
26951	CGCGGGGTCT	TTAGCCGTTG	GCCAAGGTCG	ATGGCTACAA TACCGATGTT	GGAGGCGAGG
27001	AGTCCGCGGC	GGCCGTGACG	GGCAAGCGGC	ACCCAACCGT TGGGTTGGCA	TCTACCCTGT
27051	GGTGACCTTG	GTCCCGGCCA	TTCAGGTTCG	AGCCGCCGCC TCGGCGGCGG	CAATCGGGTT
27101	CTCGTTGTTG	TCGCGGTTCC	GATGGCGAGT	TGGCGCGGGC ACCGCGCCCG	TGTTCTTGCG
27151	GTATCAACGA	ACGAACGTTC	TGACACCCCC	CAACATCTCC GTTGTAGAGG	DDDDDDDAA
27201	CGAAAGAAGA	GATGGTAGTG	CCGCACCGGA	TCCCCCGTAA AGGGGGCATT	GTAGGACGTA
27251	ATGATGGCAG	TAGAGATGTC	GGGTATGACG	TGGCCGCCGT	
		GTGTGTCTTC	GTTTCCGCTG	GCCTATCGTT	CTGAGACTGT
		TTAGGTGTCG	CCGCCGTCGT	CGTCCTCCTC	CTCGCGACGC
27401	TCTGGCGCCC AGACCGCGGG				AACAGGATTT TTGTCCTAAA
27451	TTCCCACTCT AAGGGTGAGA	GTATGCTATA CATACGATAT	TTTCAACAGA AAAGTTGTCT	GCAGGGGCCA CGTCCCCGGT	AGAACAAGAG TCTTGTTCTC

Figure 26: AC

27501	CTGAAAATAA GACTTTTATT	A CAGGTC TITTGTCCAG	TCTGCGATCC AGACGCTAGG	CTCACCCGCA GAGTGGGCGT	GCTGCC A CGACGGACAT
27551	TCACAAAAGC AGTGTTTTCG	GAAGATCAGC CTTCTAGTCG	TTCGGCGCAC AAGCCGCGTG	GCTGGAAGAC CGACCTTCTG	GCGGAGGCTC CGCCTCCGAG
27601	TCTTCAGTAA AGAAGTCATT	ATACTGCGCG TATGACGCGC	CTGACTCTTA GACTGAGAAT	AGGACTAGTT TCCTGATCAA	TCGCGCCCTT AGCGCGGGAA
27651	TCTCAAATTT AGAGTTTAAA	AAGCGCGAAA TTCGCGCTTT	ACTACGTCAT TGATGCAGTA	CTCCAGCGGC GAGGTCGCCG	CACACCCGGC GTGTGGGCCG
27701	GCCAGCACCT CGGTCGTGGA	GTTGTCAGCG CAACAGTCGC	CCATTATGAG GGTAATACTC	CAAGGAAATT GTTCCTTTAA	CCCACGCCCT GGGTGCGGGA
27751	ACATGTGGAG TGTACACCTC	TTACCAGCCA AATGGTCGGT	CAAATGGGAC GTTTACCCTG	TTGCGGCTGG AACGCCGACC	AGCTGCCCAA TCGACGGGTT
27801	GACTACTCAA CTGATGAGTT	CCCGAATAAA GGGCTTATTT	CTACATGAGC GATGTACTCG	GCGGGACCCC	ACATGATATC TGTACTATAG
27851	CCGGGTCAAC GGCCCAGTTG	GGAATACGCG CCTTATGCGC	CCCACCGAAA GGGTGGCTTT	CCGAATTCTC GGCTTAAGAG	CTGGAACAGG GACCTTGTCC
27901	CGGCTATTAC GCCGATAATG	CACCACACCT GTGGTGTGGA	CGTAATAACC GCATTATTGG	TTAATCCCCG AATTAGGGGC	TAGTTGGCCC
27951	GCTGCCCTGG CGACGGGACC	TGTACCAGGA ACATGGTCCT	AAGTCCCGCT TTCAGGGCGA	CCCACCACTG GGGTGGTGAC	TGGTACTTCC ACCATGAAGG
28001	GTCTCTGCGG	GTCCGGCTTC	TTCAGATGAC AAGTCTACTG	ATTGAGTCCC	CGCGTCGAAC
28051	GCCCGCCGAA	AGCAGTGTCC	GTGCGGTCGC CACGCCAGCG	GGCCCGTCCC	ATATTGAGTG
28101	GACTGTTAGT	CTCCCGCTCC	TATTCAGCTC ATAAGTCGAG	TTGCTGCTCA	GCCACTCGAG
28151	GAGCGAACCA	GAGGCAGGCC	ACGGGACATT TGCCCTGTAA	AGTCTAGCCG	CCGCGGCCGG
28201	CGAGAAGTAA	GTGCGGAGCA	CAGGCAATCC GTCCGTTAGG	ATTGAGACGT	CTGGAGCAGG
	AGACTCGGCG	CGAGACCTCC	GTAACCTIGA	GACGTTAAAT	TTGAGGAGTT AACTCCTCAA
28301	TGTGCCATCG ACACGGTAGC	GTCTACTTTA CAGATGAAAT	ACCCCTTCTC TGGGGAAGAG	GGGACCTCCC	GGCCACTATC CCGGTGATAG
28351	CGGATCAATT GCCTAGTTAA	TATTCCTAAC ATAAGGATTG	TTTGACGCGG AAACTGCGCC	TAAAGGACTC ATTTCCTGAG	GGCGGACGGC CCGCCTGCCG
28401	TACGACTGAA ATGCTGACTT	TGTTAAGTGG ACAATTCACC	AGAGGCAGAG TCTCCGTCTC	CAACTGCGCC	TGAAACACCT ACTTTGTGGA

Figure 26 AD

28451	•••••	• • • • • • • • • • • • • • • • • • • •		CCGCGACTCC GGCGCTGAGG	
28501				AGGGCCCGGC TCCCGGGCCG	
28551				AGCCTGATTC TCGGACTAAG	
28601				GGGACCCTGT CCCTGGGACA	
28651				ATCAAGATCT TAGTTCTAGA	
28701				TAAAATATAC ATTTTATATG	
28751				CCCGCCCAAG GGGCGGGTTC	
28801				CCCTCTGTGA GGGAGACACT	
28851				GAACCTCTCC CTTGGAGAGG	
28901				CCTGCCGGGA GGACGGCCCT	
28951				CCTGACCGTA GGACTGGCAT	
29001				CCAGAACAGG GGTCTTGTCC	
29051				GCAGCTACTG CGTCGATGAC	
29101				TAATTCAGGT ATTAAGTCCA	
29151				TTCTCTTTAT AAGAGAAATA	
29201	ACGCTTCTCT TGCGAAGAGA			TGTGTGCACA ACACACGTGT	
29251	TTGTCAGCTT AACAGTCGAA	TTTAAACGCT AAATTTGCGA	GGGGTCGCCA CCCCAGCGGT	CCCAAGATGA GGGTTCTACT	TTAGGTACAT AATCCATGTA
29301	AATCCTAGGT TTAGGATCCA			CCACGGTACC GGTGCCATGG	
29351	TGGATTTTAA ACCTAAAATT			CATTCGCAGC GTAAGCGTCG	

Figure 26 AE

29401	GAGTGCACCA	CTTATAAA	ATGCACCACA	GAACATGAAA	AGCTGU AT
	CTCACGTGGT	GAGAATATTT	TACGTGGTGT	CTTGTACTTT	TCGACGAATA
29451	TCGCCACAAA	AACAAAATTG	GCAAGTATGC	TGTTTATGCT	ATTTGGCAGC
	AGCGGTGTTT	TTGTTTTAAC	CGTTCATACG	ACAAATACGA	TAAACCGTCG
29501	CAGGTGACAC	TACAGAGTAT	AATGTTACAG	TTTTCCAGGG	TAAAAGTCAT
	GTCCACTGTG	ATGTCTCATA	TTACAATGTC	AAAAGGTCCC	ATTTTCAGTA
29551	AAAACTTTTA	TGTATACTTT	TCCATTTTAT	GAAATGTGCG	ACATTACCAT
	TTTTGAAAAT	ACATATGAAA	AGGTAAAATA	CTTTACACGC	TGTAATGGTA
29601	GTACATGAGC	AAACAGTATA	AGTTGTGGCC	CCCACAAAAT	TGTGTGGAAA
	CATGTACTCG	TTTGTCATAT	TCAACACCGG	GGGTGTTTTA	ACACACCTTT
29651	ACACTGGCAC	TTTCTGCTGC	ACTGCTATGC	TAATTACAGT	GCTCGCTTTG
	TGTGACCGTG	AAAGACGACG	TGACGATACG	ATTAATGTCA	CGAGCGAAAC
29701	GTCTGTACCC	TACTCTATAT	TAAATACAAA	AGCAGACGCA	GCTTTATTGA
	CAGACATGGG	ATGAGATATA	ATTTATGTTT	TCGTCTGCGT	CGAAATAACT
29751	GGAAAAGAAA	ATGCCTTAAT	TTACTAAGTT	ACAAAGCTAA	TGTCACCACT
	CCTTTTCTTT	TACGGAATTA	AATGATTCAA	TGTTTCGATT	ACAGTGGTGA
29801	AACTGCTTTA	CTCGCTGCTT	GCAAAACAAA	TTCAAAAAGT	TAGCATTATA
:	TTGACGAAAT	GAGCGACGAA	CGTTTTGTTT	AAGTTTTTCA	ATCGTAATAT
29851	ATTAGAATAG	GATTTAAACC	CCCCGGTCAT	TTCCTGCTCA	ATACCATTCC
	TAATCTTATC	CTAAATTTGG	GGGGCCAGTA	AAGGACGAGT	TATGGTAAGG
29901	CCTGAACAAT	TGACTCTATG	TGGGATATGC	TCCAGCGCTA	CAACCTTGAA
	GGACTTGTTA	ACTGAGATAC	ACCCTATACG	AGGTCGCGAT	GTTGGAACTT
29951	GTCAGGCTTC	CTGGATGTCA	GCATCTGACT	TTGGCCAGCA	CCTGTCCCGC
	CAGTCCGAAG	GACCTACAGT	CGTAGACTGA	AACCGGTCGT	GGACAGGGCG
30001	GGATTTGTTC	CAGTCCAACT	ACAGCGACCC	ACCCTAACAG	AGATGACCAA
	CCTAAACAAG	GTCAGGTTGA	TGTCGCTGGG	TGGGATTGTC	TCTACTGGTT
30051	CACAACCAAC GTGTTGGTTG	CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC	CTACCGGACT GATGGCCTGA	TACATCTACC ATGTAGATGG	ACAAATACAC TGTTTATGTG
30101	CCCAAGTTTC	TGCCTTTGTC	AATAACTGGG	ATAACTTGGG	CATGTGGTGG
	GGGTTCAAAG	ACGGAAACAG	TTATTGACCC	TATTGAACCC	GTACACCACC
30151	TTCTCCATAG	CGCTTATGTT	TGTATGCCTT	ATTATTATGT	GGCTCATCTG
	AAGAGGTATC	GCGAATACAA	ACATACGGAA	TAATAATACA	CCGAGTAGAC
30201	CTGCCTAAAG	CGCAAACGCG	CCCGACCACC	CATCTATAGT	CCCATCATTG
	GACGGATTTC	GCGTTTGCGC	GGGCTGGTGG	GTAGATATCA	GGGTAGTAAC
30251	TGCTACACCC	AAACAATGAT	GGAATCCATA	GATTGGACGG	ACTGAAACAC
	ACGATGTGGG	TTTGTTACTA	CCTTAGGTAT	CTAACCTGCC	TGACTTTGTG
30301	ATGTTCTTTT	CTCTTACAGT	ATGATTAAAT	GAGACATGAT	TCCTCGAGTT
	TACAAGAAAA	GAGAATGTCA	TACTAATTTA	CTCTGTACTA	AGGAGCTCAA

Figure 26 AF

30351	TTTATATTAC AAATATAATG				CCACAT C GGTGTAACCG
30401		CACATCGAAG GTGTAGCTTC			
30451		ATTTGTCACC TAAACAGTGG			
30501		TTATCCAGTG AATAGGTCAC			
30551		CATCCCCAGT GIAGGGGTCA			
30601		ATTATGAAAT TAATACTTTA			
30651		GTTTTGTTCC CAAAACAAGG			
30701		CTCGTATATG GAGCATATAC			
30751	• • • • • • • • •	GAAGCCTGGT CTTCGGACCA			
30801		CTTAGCCCTA GAATCGGGAT	-	-	
30851		ATGCCATGAA TACGGTACTT			
30901		CAAGTTGTTG GTTCAACAAC			
30951		TCCCACCCCC AGGGTGGGGG			
31001		GACACCCTAG CTGTGGGATC			
31051		AGAAAGACGC TCTTTCTGCG			
31101	CAAGAGCTCC GTTCTCGAGG	AAGACATGGT TTCTGTACCA			
31151	TTGTCTCGTA AACAGAGCAT	AAGCAGGCCA TTCGTCCGGT			
31201	ACCGCCTTAG TGGCGGAATC	CTACAAGTTG GATGTTCAAC			
31251	GTGGGAGAAA CACCCTCTTT	AGCCCATTAC TCGGGTAATG			

Figure 26 AG

31301 -	CTGCATTCAC	TCTTGTC	AAGGACCTGA	GGATCTCTGC	ACCCTT TA
	GACGTAAGTG	AGTGGAACAG	TTCCTGGACT	CCTAGAGACG	TGGGAALAT
31351	AGACCCTGTG	CGGTCTCAAA	GATCTTATTC	CCTTTAACTA	ATAAAAAAAA
	TCTGGGACAC	GCCAGAGTTT	CTAGAATAAG	GGAAATTGAT	TATTTTTTAT
31401	ATAATAAAGC	ATCACTTACT	TAAAATCAGT	TAGCAAATTT	CTGTCCAGTT
	TATTATTTCG	TAGTGAATGA	ATTTTAGTCA	ATCGTTTAAA	GACAGGTCAA
31451	TATTCAGCAG	CACCTCCTTG	CCCTCCTCCC	AGCTCTGGTA	TTGCAGCTTC
	ATAAGTCGTC	GTGGAGGAAC	GGGAGGAGGG	TCGAGACCAT	AACGTCGAAG
31501	CTCCTGGCTG	CAAACTTTCT	CCACAATCTA	AATGGAATGT	CAGTTTCCTC
	GAGGACCGAC	GTTTGAAAGA	GGTGTTAGAT	TTACCTTACA	GTCAAAGGAG
31551	CTGTTCCTGT	CCATCCGCAC	CCACTATCTT	CATGTTGTTG	CAGATGAAGC
	GACAAGGACA	GGTAGGCGTG	GGTGATAGAA	GTACAACAAC	GTCTACTTCG
31601	GCGCAAGACC	GTCTGAAGAT	ACCTTCAACC	CCGTGTATCC	ATATGACACG
	CGCGTTCTGG	CAGACTTCTA	TGGAAGTTGG	GGCACATAGG	TATACTGTGC
31651	GAAACCGGTC	CTCCAACTGT	GCCTTTTCTT	ACTCCTCCCT	TTGTATCCCC
	CTTTGGCCAG	GAGGTTGACA	CGGAAAAGAA	TGAGGAGGGA	AACATAGGGG
31701	GTTACCCAAA	GTTCTCTCAG	GGGGACCCCA	ACTCTCTTTG TGAGAGAAAC	GCGGATAGGC
31751	AACCTCTAGT	TACCTCCAAT	GGCATGCTTG	CGCTCAAAAT	GGGCAACGGC
	TTGGAGATCA	ATGGAGGTTA	CCGTACGAAC	GCGAGTTTTA	CCCGTTGCCG
31801	GAGAGAGACC	TGCTCCGGCC	GTTGGAATGG	TCCCAAAATG CATTTTDDDAA	ATTGGTGACA
31851	CTCGGGTGGA	GAGTTTTTT	GGTTCAGTTT	CATAAACCTG GTATTTGGAC	CTTTATAGAC
31901	GTGGGGAGTG	TCAATGGAGT	CTTCGGGATT	GACACCGACG	CGCCGCACCT GCGGCGTGGA
31951	GATTACCAGC	GCCCGTTGTG	TGAGTGGTAC	GTTAGTGTCC	CCCCGCTAAC GGGGCGATTG
32001	GCACGTGCTG	AGGTTTGAAT	CGTAACGGTG	GGTTCCTGGG	CTCACAGTGT GAGTGTCACA
	GTCTTCCTTT	CGATCGGGAC	GTTTGTAGTC	CGGGGGAGTG	CACCACCGAT GTGGTGGCTA
	TCGTCATGGG	AATGATAGTG	ACGGAGTGGG	GGAGATTGAT	CTGCCACTGG
	ATCGAACCCG	TAACTGAACT	TTCTCGGGTA	AATATGTGTT	AATGGAAAAC TTACCTTTTG
32201	TAGGACTAAA	GTACGGGGCT	CCTTTGCATG	TAACAGACGA	CCTAAACACT
	ATCCTGATTT	CATGCCCCGA	GGAAACGTAC	ATTGTCTGCT	GGATTTGTGA

Figure 26 AH

32251	TTGACCGTAG AACTGGCATC	CTGGTCC GACCAGG	AGGTGTGACT TCCACACTGA	ATKATAATTA TATTATTAAT	CTTCCTPCA GAAGGA GT
32301		ACTGGAGCCT TGACCTCGGA			
32351		AGGAGGACTA TCCTCCTGAT			
32401		GTTATCCGTT CAATAGGCAA		_	
32451		CCTCTTTTTA GGAGAAAAAT			
32501		CCTTTACTTG GGAAATGAAC			
32551		TAAGCACTGC ATTCGTGACG			
32601		GCAGGAGATG CGTCCTCTAC			
32651		CCTCAAAACA GGAGTTTTGT			
32701		TGGTTCCTAA ACCAAGGATT			
32751		ACAGTAGGAA TGTCATCCTT			
32801		TCCATCTCCT AGGTAGAGGA			
32851		TGGTCTTAAC ACCAGAATTG			
32901		GCTGTTAAAG CGACAATTTC			
32951		TCTTATTATA AGAATAATAT			
33001	AATTCCTTCC TTAAGGAAGG	TGGACCCAGA ACCTGGGTCT	ATATTGGAAC TATAACCTTG	TTTAGAAATG AAATCTTTAC	GAGATCTTAC CTCTAGAATG
33051	TGAAGGCACA ACTTCCGTGT	GCCTATACAA CGGATATGTT	ACGCTGTTGG TGCGACAACC	ATTTATGCCT TAAATACGGA	AACCTATCAG TTGGATAGTC
33101	CTTATCCAAA GAATAGGTTT	ATCTCACGGT TAGAGTGCCA			
33151	GTTTACTTAA CAAATGAATT	ACGGAGACAA TGCCTCTGTT			

Figure 26 AI

33201	AAACGGTACA TTTGCCATGT	GALCTTTGTC	GAGACACAAC CTCTGTGTTG	TCCAAGTGCA AGGTTCACGT	TACTOT T ATGAGACA
33251	CATTTTCATG	GGACTGGTCT	GGCCACAACT	ACATTAATGA	AATATTTGCC
	GTAAAAGTAC	CCTGACCAGA	CCGGTGTTGA	TGTAATTACT	TTATAAACGG
33301	ACATCCTCTT	ACACTTTTTC	ATACATTGCC	CAAGAATAAA	GAATCGTTTG
	TGTAGGAGAA	TGTGAAAAAG	TATGTAACGG	GTTCTTATTT	CTTAGCAAAC
33351	TGTTATGTTT	CAACGTGTTT	ATTTTTCAAT	TGCAGAAAAT	TTCAAGTCAT
	ACAATACAAA	GTTGCACAAA	TAAAAAGTTA	ACGTCTTTTA	AAGTTCAGTA
33401	TTTTCATTCA	GTAGTATAGC	CCCACCACCA	CATAGCTTAT	ACAGATCACC
	AAAAGTAAGT	CATCATATCG	GGGTGGTGGT	GTATCGAATA	TGTCTAGTGG
33451	GTACCTTAAT CATGGAATTA	CAAACTCACA GTTTGAGTGT	GAACCCTAGT CTTGGGATCA	ATTCAACCTG TAAGTTGGAC	CCACCTCCCT
33501	CCCAACACAC	AGAGTACACA	GTCCTTTCTC	CCCGGCTGGC	CTTAAAAAGC
	GGGTTGTGTG	TCTCATGTGT	CAGGAAAGAG	GGGCCGACCG	GAATTTTTCG
33551	ATCATATCAT	GGGTAACAGA	CATATTCTTA	GGTGTTATAT	TCCACACGGT
	TAGTATAGTA	CCCATTGTCT	GTATAAGAAT	CCACAATATA	AGGTGTGCCA
33601	TTCCTGTCGA	GCCAAACGCT	CATCAGTGAT	ATTAATAAAC	TCCCCGGGCA
	AAGGACAGCT	CGGTTTGCGA	GTAGTCACTA	TAATTATTTG	AGGGGCCCGT
33651	GCTCACTTAA	GTTCATGTCG	CTGTCCAGCT	GCTGAGCCAC	AGGCTGCTGT
	CGAGTGAATT	CAAGTACAGC	GACAGGTCGA	CGACTCGGTG	TCCGACGACA
33701	CCAACTTGCG	GTTGCTTAAC	GGGCGGCGAA	GGAGAAGTCC	ACGCCTACAT
	GGTTGAACGC	CAACGAATTG	CCCGCCGCTT	CCTCTTCAGG	TGCGGATGTA
33751	GGGGGTAGAG	TCATAATCGT	GCATCAGGAT	AGGGCGGTGG	TGCTGCAGCA
	CCCCCATCTC	AGTATTAGCA	CGTAGTCCTA	TCCCGCCACC	ACGACGTCGT
33801	GCGCGCGAAT CGCGCGCTTA	AAACTGCTGC TTTGACGACG	CGCCGCCGCGA	CCGTCCTGCA GGCAGGACGT	GGAATACAAC CCTTATGTTG
33851	ATCCCAGTCG	TCTCCTCAGC	GATGATTCGC	ACCGCCCGCA	GCATAAGGCG
	TACCGTCACC	AGAGGAGTCG	CTACTAAGCG	TGGCGGGCGT	CGTATTCCGC
33901	CCTTGTCCTC	CGGGCACAGC	AGCGCACCCT	GATCTCACTT	AAATCAGCAC
	GGAACAGGAG	GCCCGTGTCG	TCGCGTGGGA	CTAGAGTGAA	TTTAGTCGTG
33951	AGTAACTGCA	GCACAGCACC	ACAATATTGT	TCAAAATCCC	ACAGTGCAAG
	TCATTGACGT	CGTGTCGTGG	TGTTATAACA	AGTTTTAGGG	TGTCACGTTC
34001	GCGCTGTATC	CAAAGCTCAT	GGCGGGGACC	ACAGAACCCA	CGTGGCCATC
	CGCGACATAG	GTTTCGAGTA	CCGCCCTGG	TGTCTTGGGT	GCACCGGTAG
34051	ATACCACAAG	CGCAGGTAGA	TTAAGTGGCG	ACCCCTCATA	AACACGCTGG
	TATGGTGTTC	GCGTCCATCT	AATTCACCGC	TGGGGAGTAT	TTGTGCGACC
34101	ACATAAACAT	TACCTCTTTT	GGCATGTTGT	AATTCACCAC	CTCCCGGTAC
	TGTATTTGTA	ATGGAGAAAA	CCGTACAACA	TTAAGTGGTG	GAGGGCCATG

Figure 26 AJ

34151	CATATAAACC GTATATTTGG	T CATTAAA A CTAATTT	CATGGCGCCA GTACCGCGGT	TCCACCACTAT AGGTGGTGGT	AGGATT ET
34201				CTGCAGGGAA GACGTCCCTT	
34251				AACCATGGAT TTGGTACCTA	
34301				CACACGTGCA GTGTGCACGT	
34351	CAGGATTACA GTCCTAATGT			CATATCCCAG GTATAGGGTC	
34401				AGGGAAGACC TCCCTTCTGG	
34451	GAGTGCAACA	CGTAACAGTT	TCACAATGTA	TCGGGCAGCA AGCCCGTCGT	CGCCTACTAG
34501	GAGGTCATAC	CATCGCGCCC	AAAGACAGAG	AAAAGGAGGT TTTTCCTCCA	TCTGCTAGGG
34551		TCACGCGGCT	CTGTTGGCTC	TAGCACAACC	AGCATCACAG
34601				TTTCCTGAAG AAAGGACTTC	
34651				GGTCTCGCCG CCAGAGCGGC	
34701	AGACACATCA	TCAACATCAT	ATAGGTGAGA	CTCAAAGCAT GAGTTTCGTA	GGTCCGCGGG
34751	GGACCGAAGC	CCAAGATACA	TTTGAGGAAG	ATGCGCCGCT TACGCGGCGA	CGGGACTATT
34801	GTAGGTGGTG	GCGTCTTATT	CGGTGTGGGT	GCCAACCTAC CGGTTGGATG	TGTAAGCAAG
34851	ACGCTCAGTG	TGTGCCCTCC	TCGCCCTTCT	GCTGGAAGAA CGACCTTCTT	GGTACAAAAA
		GTTTTCTAAT	AGGTTTTGGA	GTTTTACTTC	TAGATAATTC
		GGGGAGGCCA	CCGCACCAGT	TTGAGATGTC	GCTTTCTTCT
		AAACATTCTA	CAACGTGTTA	CCGAAGGTTT	TCCGTTTGCC
35051	CCCTCACGTC GGGAGTGCAG	CAAGTGGACG GTTCACCTGC	TAAAGGCTAA ATTTCCGATT	ACCCTTCAGG TGGGAAGTCC	GTGAATCTCC CACTTAGAGG

Figure 26AK

35101	TCTATAAACA AGATATTTGT	TAGCACC AAGGTCGTGG	TTCAACCATG AAGTTGGTAC	CCCAAATAAT GGGTTTATTA	TCTCAT G AGAGTAGAGC
35151	CCACCTTCTC GGTGGAAGAG			CCGAATATTA GGCTTATAAT	
35201				CCTTCAGCCT GGAAGTCGGA	
35251				AGACCTGTAT TCTGGACATA	
35301	AGCGGAACAT TCGCCTTGTA	ТААСАААААТ АТТСТТТТА	ACCGCGATCC TGGCGCTAGG	CGTAGGTCCC GCATCCAGGG	TTCGCAGGGC AAGCGTCCCG
35351	CAGCTGAACA GTCGACTTGT	TAATCGTGCA ATTAGCACGT	GGTCTGCACG CCAGACGTGC	GACCAGCGCG CTGGTCGCGC	GCCACTTCCC CGGTGAAGGG
35401	CGCCAGGAAC GCGGTCCTTG	CATGACAAAA GTACTGTTTT	GAACCCACAC CTTGGGTGTG	TGATTATGAC ACTAATACTG	ACGCATACTC TGCGTATGAG
35451	GGAGCTATGC CCTCGATACG	TAACCAGCGT ATTGGTCGCA	AGCCCCGATG TCGGGGCTAC	TAAGCTTGTT ATTCGAACAA	GCATGGGCGG CGTACCCGCC
35501				ATCAGGCAAA TAGTCCGTTT	
35551	TTTTTCTTTC	GTGTAGCATC	AGTACGAGTA	GCAGATAAAG CGTCTATTTC	CGTCCATTCG
35601	TCCGGAACCA AGGCCTTGGT	CCACAGAAAA GGTGTCTTTT	AGACACCATT TCTGTGGTAA	TTTCTCTCAA AAAGAGAGTT	ACATGTCTGC TGTACAGACG
35651	CCCAAAGACG	TATTTGTGTT	TTATTTTATT	CAAAAAAACA GTTTTTTGT	AAATTTGTAA
35701	TCTTCGGACA	GAATGTTGTC	CTTTTTGTTG	CCTTATAAGC GGAATATTCG	TATTCTGCCT
35751	GATGCCGGTA	CGGCCGCACT	GGCATTTTTT	ACTGGTCACC TGACCAGTGG	CACTAATTTT
35801	TCGTGGTGGC	TGTCGAGGAG	CCAGTACAGG	GGAGTCATAA CCTCAGTATT	ACATTCTGAG
		AGTCCAACTA	AGTGTAGCCA	GTCACGATTT	TTCGCTGGCT
		CCCTTATGTA	TGGGCGTCCG	CATCTCTGTT	GTAATGTCGG
		CATATTGTTT	TAATTATCCT	CTCTTTTTGT	GTATTTGTGG
36001	TGAAAAACCC ACTTTTTGGG	TCCTGCCTAG AGGACGGATC	GCAAAATAGC CGTTTTATCG	ACCCTCCCGC TGGGAGGGCG	TCCAGAACAA AGGTCTTGTT

Figure 26 AL

36051	CATACAGCGC GTATGTCGCG	ACAGCG AAGGTGTCGC	GCAGCCATAA CGTCGGTATT	CAGTCAGCCT GTCAGTCGGA	TACCAG A
36101				ACACGGCACC TGTGCCGTGG	
36151	GTCACAGTGT CAGTGTCACA	AAAAAAGGGC TTTTTTCCCG	CAAGTGCAGA GTTCACGTCT	GCGAGTATAT CGCTCATATA	ATAGGACTAA TATCCTGATT
36201				AACACCCAGA TTGTGGGTCT	
36251	CGAACCTACG GCTTGGATGC	CCCAGAAACG GGGTCTTTGC	AAAGCCAAAA TTTCGGTTTT	AACCCACAAC TTGGGTGTTG	TTCCTCAAAT AAGGAGTTTA
36301	CGTCACTTCC GCAGTGAAGG	GTTTTCCCAC CAAAAGGGTG	GTTACGTCAC CAATGCAGTG	TTCCCATTIT AAGGGTAAAA	AAGAAAACTA TTCTTTTGAT
36351	CAATTCCCAA GTTAAGGGTT	CACATACAAG GTGTATGTTC	TTACTCCGCC AATGAGGCGG	CTAAAACCTA GATTTTGGAT	CGTCACCCGC GCAGTGGGCG
36401	CCCGTTCCCA GGGCAAGGGT	GCGCCCGCGC	CACGTCACAA GTGCAGTGTT	ACTCCACCCC TGAGGTGGGG	CTCATTATCA GAGTAATAGT
					PacI
36451	TATTGGCTTC	AATCCAAAAT	AAGGTATATT	ATTGATGATG	TTAATTAAGA
	ATAACCGAAG	TTAGGTTTTA	TTCCATATAA	TAACTACTAC	AATTAATTCT
36501	ATTCGGATCT	GCGACGCGAG	GCTGGATGGC	CTTCCCCATT	ATGATTCTTC
	TAAGCCTAGA	CGCTGCGCTC	CGACCTACCG	GAAGGGGTAA	TACTAAGAAG
36551	TCGCTTCCGG	CGGCATCGGG	ATGCCCGCGT	TGCAGGCCAT	GCTGTCCAGG
	AGCGAAGGCC	GCCGTAGCCC	TACGGGCGCA	ACGTCCGGTA	CGACAGGTCC
36601				CAAGGCCAGC	
	GTCCATCTAC	TGCTGGTAGT	CCCTGTCGAA	GTTCCGGTCG	TTTTCCGGTC
36651				TTTCCATAGG	
	CTTGGCATTT	TTCCGGCGCA	ACGACCGCAA	AAAGGTATCC	GAGGCGGGGG
36701				GTCAGAGGTG	
	GACTGCTCGT	AGTGTTTTTA	GCTGCGAGTT	CAGTCTCCAC	CGCTTTGGGC
36751	ACAGGACTAT	AAAGATACCA	GGCGTTTCCC	CCTGGAAGCT	CCCTCGTGCG
	TGTCCTGATA	TTTCTATGGT	CCGCAAAGGG	GGACCTTCGA	GGGAGCACGC
36801	CTCTCCTGTT	CCGACCCTGC	CGCTTACCGG	ATACCTGTCC	GCCTTTCTCC
	GAGAGGACAA	GGCTGGGACG	GCGAATGGCC	TATGGACAGG	CGGAAAGAGG
36851	CTTCGGGAAG	CGTGGCGCTT	TCTCATAGCT	CACGCTGTAG	GTATCTCAGT
•	GAAGCCCTTC	GCACCGCGAA	AGAGTATCGA	GTGCGACATC	CATAGAGTCA
36901	TCGGTGTAGG	TCGTTCGCTC	CAAGCTGGGC	TGTGTGCACG	AACCCCCCGT
	AGCCACATCC	AGCAAGCGAG	GTTCGACCCG	ACACACGTGC	TTGGGGGGCA

Figure 26 AM

36951	TCAGCCCGAC AGTCGGGCTG	TGCGCCT GCGACGCGGA	TATCCGGTAA ATAGGCCATT	CTATCGTCTT GATAGCAGAA	GAGTCO TC CTCAGG. GG
37001	CGGTAAGACA GCCATTCTGT	CGACTTATCG GCTGAATAGC	CCACTGGCAG GGTGACCGTC	CAGCCACTGG GTCGGTGACC	TAACAGGATT ATTGTCCTAA
37051	TCGTCTCGCT	CCATACATCC	CGGTGCTACA GCCACGATGT	CTCAAGAACT	TCACCACCGG
37101	ATTGATGCCG	ATGTGATCTT	GGACAGTATT CCTGTCATAA	ACCATAGACG	CGAGACGACT
37151	TCGGTCAATG	GAAGCCTTTT	AGAGTTGGTA TCTCAACCAT	CGAGAACTAG	GCCGTTTGTT
37201	TGCTGGCGAC	CATCGCCACC	TTTTTTTGTT AAAAAAACAA	ACGTTCGTCG	TCTAATGCGC
37251	GTCTTTTTT	CCTAGAGTTC	AAGATCCTTT TTCTAGGAAA	CTAGAAAAGA	TGCCCCAGAC
37301	TGCGAGTCAC	CTTGCTTTTG	TCACGTTAAG AGTGCAATTC	CCTAAAACCA	GTACTCTAAT
37351	AGTTTTTCCT	AGAAGTGGAT	GATCCTTTTA CTAGGAAAAT	TTAGTTAGAT	TTCATATATA
37401	CTCATTTGAA	CCAGACTGTC	TTACCAATGC AATGGTTACG	AATTAGTCAC	TCCGTGGATA
37451	GAGTCGCTAG	ACAGATAAAG	GTTCATCCAT CAAGTAGGTA	TCAACGGACT	GAGGGGCAGC
37501	ACATCTATTG	ATGCTATGCC	GAGGGCTTAC CTCCCGAATG	GTAGACCGGG	GTCACGACGT
37551	TACTATGGCG	CTCTGGGTGC	CTCACCGGCT GAGTGGCCGA	GGTCTAAATA	GTCGTTATTT
37601	GGTCGGTCGG	CCTTCCCGGC	AGCGCAGAAG TCGCGTCTTC	ACCAGGACGT	TGAAATAGGC
37651	GGAGGTAGGT	CAGATAATTA	TGTTGCCGGG ACAACGGCCC	TTCGATCTCA	TTCATCAAGC
	GGTCAATTAT	CAAACGCGTT	GCAACAACGG	TAACGATGTC	GCATCGTGGT CGTAGCACCA
		AGCAAACCAT	ACCGAAGTAA	GTCGAGGCCA	AGGGTTGCTA
		ATGTACTAGG	GGGTACAACA	CGTTTTTTCG	CCAATCGAGG
37851	TTCGGTCCTC AAGCCAGGAG	CGATCGTTGT GCTAGCAACA	CAGAAGTAAG GTCTTCATTC	TTGGCCGCAG	TGTTATCACT ACAATAGTGA

Figure 26 AN

37901	CATGGTTATG GTACCAATAC	CELCGTGACG	ATAATTCTCT TATTAAGAGA	TACTGTCATG ATGACAGTAC	CCATCO TAA GGTAGGCATT
37951	GATGCTTTTC CTACGAAAAG	TGTGACTGGT ACACTGACCA	GAGTACTCAA CTCATGAGTT	CCAAGTCATT GGTTCAGTAA	CTGAGAATAG GACTCTTATC
38001				GCGTCAACAC CGCAGTTGTG	
38051				CATCATTGGA GTAGTAACCT	
38101				TGTTGAGATC ACAACTCTAG	
38151				GCATCTTTTA CGTAGAAAAT	
38201				AAATGCCGCA TTTACGGCGT	
38251				TACTCTTCCT ATGAGAAGGA	
38301				ATGAGCGGAT TACTCGCCTA	
38351				TCCGCGCACA AGGCGCGTGT	
38401				TTATCATGAC AATAGTACTG	
38451	AAAAATAGGC TTTTTATCCG			CTTCAAGAAT GAAGTTCTTA	
		PacI			
38501	TTCTTAATTT AAGAATTAAA	CTTAATTAA GAATTAATT			

Figure 26 AO

MRKAd5nef MER1063 (MRKAd5 Pre-Adenoviral Vector Containing the G2A,LLA nef Coding Region)

1	CATCATCAAT	AATATACCTT	ATTTTGGATT	GAAGCCAATA	TGATAATGAG
	GTAGTAGTTA	TTATATGGAA	TAAAACCTAA	CTTCGGTTAT	ACTATTACTC
	•				
51	GGGGTGGAGT	TTGTGACGTG	GCGCGGGGG	TGGGAACGGG	GCGGGTGACG
	CCCCACCTCA	AACACTGCAC	Cececccec	ACCCTTGCCC	CGCCCACTGC
	•	•			
101	TAGTAGTGTG	GCGGAAGTGT	GATGTTGCAA	GTGTGGCGGA	ACACATGTAA
	ATCATCACAC	CGCCTTCACA	CTACAACGTT	CACACCGCCT	TGTGTACATT
	GCGACGGATG		a. 00mmmm		СПСТВСВСВС
151	GCGACGGATG	TGGCAAAAGT	CTGCAAAAAC	CACACGCGG	CACATGTGTC
	CGCTGCCTAC	ACCGITITCA	CIGCNAMAC	CACACGCGGG	C.1.C.1.101010
201	CARCTGRCAR	יייייירוברפרפ	GTTTTAGGCG	GATGTTGTAG	TAAATTTGGG
201	CTTC & CTCTT	AAAAGCGCGC	CAAAATCCGC	CTACAACATC	ATTTAAACCC
	CIICACIGII	***************************************			
251	CGTAACCGAG	TAAGATTTGG	CCATTTTCGC	GGGAAAACTG	AATAAGAGGA
	GCATTGGCTC	ATTCTAAACC	GGTAAAAGCG	CCCTTTTGAC	TTATTCTCCT
301	AGTGAAATCT	GAATAATTTT	GTGTTACTCA	TAGCGCGTAA	TATTTGTCTA
	TCACTTTAGA	CTTATTAAAA	CACAATGAGT	ATCGCGCATT	ATAAACAGAT
351	GGGCCGCGGG	GACTTTGACC	GTTTACGTGG	AGACTCGCCC	AGGTGTTTTT
	CCCGGCGCCC	CTGAAACTGG	CAAATGCACC	TCTGAGCGGG	TCCACAAAAA
		rmcccccmmc	CCCCTCTTTTTT	THE CONTRACT OF THE PROPERTY O	ATTATTATAG
401	CTCAGGTGTT	TICCGCGTTC	CCCCACTTTC	AACCGCAAAA	TAATAATATC
	GAGTCCACAA	AAGGCGCAAG	GCCCAGIIIC	MCCGCMPM	
451	GCGGCGGA	TCCATTGCAT	ACGTTGTATC	CATATCATAA	TATGTACATT
	CGCCGGCGCT	AGGTAACGTA	TGCAACATAG	GTATAGTATT	ATACATGTAA
501	TATATTGGCT	CATGTCCAAC	ATTACCGCCA	TGTTGACATT	GATTATTGAC
	ATATAACCGA	GTACAGGTTG	TAATGGCGGT	ACAACTGTAA	CTAATAACTG
551	TAGTTATTAA	TAGTAATCAA	TTACGGGGTC	ATTAGTTCAT	AGCCCATATA
	ATCAATAATT	ATCATTAGTT	AATGCCCCAG	TAATCAAGTA	TCGGGTATAT
		000000000000000000000000000000000000000	COMPACCOUNT	אתיכברברבר	TGGCTGACCG
601	TGGAGTTCCG	CGTTACATAA	CITACGGIAA	TACCGGGGGG	ACCGACTGGC
	ACCTCAAGGC	GCAAIGIAII	GANIGUCATI	17000000	
651	CCCAACGACC	CCCGCCCATT	GACGTCAATA	ATGACGTATG	TTCCCATAGT
002	GGGTTGCTGG	GGGCGGGTAA	CTGCAGTTAT	TACTGCATAC	AAGGGTATCA
701	AACGCCAATA	GGGACTTTCC	ATTGACGTCA	ATGGGTGGAG	TATTTACGGT
	TTGCGGTTAT	CCCTGAAAGG	TAACTGCAGT	TACCCACCTC	ATAAATGCCA
751	AAACTGCCCA	CTTGGCAGTA	CATCAAGTGT	ATCATATGCC	AAGTACGCCC
	TTTGACGGGT	GAACCGTCAT	GTAGTTCACA	TAGTATACGG	TTCATGCGGG
		max x max aca	መን እ አውድርርር	CCCWCCCAMP	ATGCCCAGTA
801	CCTATTGACG	TCAATGACGG	ATTTACCCC	CCCIGCCAII	TACGGGTCAT
	GGATAACTUC	WATTWATA	MATTIMOCOGG	COGNICCOLINA	

Figure 27A

851	CATGACCTTA GTACTGGAAT	TEACTTTC ACCTGAAAG	CTACTTGGCA GATGAACCGT	GTACATCTÃC CATGTAGATG	GTATTA CA CATAATCAGT
901			CGGTTTTGGC GCCAAAACCG		
951			ATTTCCAAGT TAAAGGITCA		
1001			AAAATCAACG TTTTAGITGC		
1051			CAAATGGGCG GTTTACCCGC		
1101			TTTAGTGAAC AAATCACTTG		
1151			TCCATAGAAG AGGTATCTTC		
1201			ATTGGAACGC TAACCTTGCG		
1251			GCAAGTGGTC CGTTCACCAG		
1301			ATGAGGAGGG TACTCCTCCC		
1351	GTGAGGAGGA CACTCCTCCT		CGCAGTGGGC GCGTCACCCG		
1401			TCACCTCCTC AGTGGAGGAG		
1451			GCCCAGGAGG CGGGTCCTCC		
1501			GAGGCCCATG CTCCGGGTAC		
1551			AGAAGGGCGG TCTTCCCGCC		
1601	CCCAGAAGAG GGGTCTTCTC	GCAGGACATC CGTCCTGTAG	CTGGACCTGT GACCTGGACA	GGGTGTACCA CCCACATGGT	CACCCAGGGC GTGGGTCCCG
1651		ACTGGCAGAA TGACCGTCTT	CTACACCCCC GATGTGGGGG	GGCCCGGCA	TCAGGTTCCC AGTCCAAGGG
1701	CCTGACCTTC GGACTGGAAG	GGCTGGTGCT CCGACCACGA	TCAAGCTGGT AGTTCGACCA	GCCCGTGGAG CGGGCACCTC	CCCGAGAAGG GGGCTCTTCC
1751	TGGAGGAGGC ACCTCCTCCG	CAACGAGGGC GTTGCTCCCG	GAGAACAACT CTCTTGTTGA	GCGCCGCCCA CGCGGCGGGT	CCCCATGTCC GGGGTACAGG

Figure 27B

1801	CAGCACGGCA GTCGTGCCGT	TAGGACCC AGCTCCTGGG	CGAGAAGGAG GCTCTTCCTC	GTGCTGGAGT CACGACCTCA	GGAGGT FA CCTCCAAGCT
1851	CTCCAAGCTG GAGGTTCGAC	GCCTTCCACC CGGAAGGTGG	ACGTGGCCAG TGCACCGGTC	GGAGCTGCAC CCTCGACGTG	CCCGAGTACT GGGCTCATGA
1901				CTGTGCCTTC GACACGGAAG	
1951	CCATCTGTTG GGTAGACAAC	TTTGCCCCTC AAACGGGGAG	CCCCGTGCCT GGGGCACGGA	TCCTTGACCC AGGAACTGGG	TGGAAGGTGC ACCTTCCACG
2001				GGAAATTGCA CCTTTAACGT	
2051				GGGTGGGGCA CCCACCCGT	
2101	CCCCTCCTAA	CCCTTCTGTT	ATCGTCCGTA	GCTGGGGATG CGACCCCTAC	GCCACCCGAG
2151	ATACCGGCTA	GCCGCGCGGC	ATGACTTTAC	TGTGGGCGTG ACACCCGCAC	CGAATTCCCA
2201	CCCTTTCTTA	TATATTCCAC	CCCCAGAATA	GTAGTTTTGT CATCAAAACA	TAGACAAAAC
2251	GTCGTCGGCG	GCGGCGGTAC	TCGTGGTTGA	CGTTTGATGG GCAAACTACC	TTCGTAACAC
2301				TGGGCCGGG ACCCGGCCCC	
2351	ACACTACCCG	AGGTCGTAAC	TACCAGCGGG	CGTCCTGCCC GCAGGACGGG	CGTTTGAGAT
2401	GATGGAACTG	GATGCTCTGG	CACAGACCTT	CGCCGTTGGA GCGGCAACCT	CTGACGTCGG
2451	AGGCGGCGGC	GAAGTCGGCG	ACCTCGCTGG	GCCCGCGGA CGGGCGCCT	AACACTGACT
2501	GAAACGAAAG	GACTCGGGCG	AACGTTTGTC	TGCAGCTTCC ACGTCGAAGG	GCAAGTAGGC
		GTTCAACTGC	CGAGAAAACC	GTGTTAACCT	AAGAAACTGG
2601	CGGGAACTTA GCCCTTGAAT	ATGTCGTTTC TACAGCAAAG	TCAGCAGCTG AGTCGTCGAC	TTGGATCTGC AACCTAGACG	GCCAGCAGGT CGGTCGTCCA
2651	TTCTGCCCTG AAGACGGGAC	AAGGCTTCCT TTCCGAAGGA	CCCCTCCCAA GGGGAGGGTT	TGCGGTTTAA ACGCCAAATT	AACATAAATA TTGTATTTAT
2701	AAAAACCAGA TTTTTGGTCT				TTGCTGTCTT AACGACAGAA

Figure 27C

2751	TATTTAGGGG ATAAATCCCC	TTTTGCGCGC AAAACGCGCG	GCGGTAGGCC CGCCATCCGG	CGGGACCAGC GCCCTGGTCG	GGTCTCTGTC CCAGAGCCAG
2801	GTTGAGGGTC CAACTCCCAG	CTGTGTATTT GACACATAAA	TTTCCAGGAC AAAGGTCCTG	GTGGTAAAGG CACCATTTCC	TGACTCTGGA ACTGAGACCT
2851		CATGGGCATA GTACCCGTAT			
2901		CATGCTGCGG GTACGACGCC			
2951	GGAGCGCTGG CCTCGCGACC	GCGTGGTGCC CGCACCACGG			
3001	GGTCCCCGTC	GCCCTTGGTG CGGGAACCAC	ATTCACAAAT	GTTTCGCCAA	TTCGACCCTA
		ÇACCCCTATA	CTCTACGTAG	AACCTGACAT	AAAAATCCAA
		GGTCGGTATA	GGGAGGCCCC	TAAGTACAAC	ACGTCTTGGT
		CATAGGCCAC	GTGAACCCTT	TAAACAGTAC	ATCGAATCTT
		CCTTCTTGAA	CCTCTGCGGG	AACACTGGAG	GTTCTAAAAG
3251	CATGCATTCG GTACGTAAGC	TCCATAATGA AGGTATTACT	TGGCAATGGG ACCGTTACCC	CCCACGGGCG GGGTGCCCGC	GCGGCCTGGG CGCCGGACCC
3301	GCTTCTATAA	TCTGGGATCA AGACCCTAGT	GATTGCAGTA	TCAACACAAG	GTCCTACTCT
3351	TCGTCATAGG AGCAGTATCC	CCATTTTTAC GGTAAAAATG			
3401	ATATTACCAA	CCATCCGGCC GGTAGGCCGG	GTCCCCGCAT	CAATGGGAGT	GTCTAAACGT
3451	AAAGGGTGCG	TTTGAGTTCA AAACTCAAGT	CTACCCCCT	AGTACAGATG	GACGCCCCGC
	TACTTCTTTT	GCCAAAGGCC	CCATCCCCTC	TAGTCGACCC	AAGAAAGCAG TTCTTTCGTC
	CAAGGACTCG	TCGACGCTGA	ATGGCGTCGG	CCACCCGGGC	TAAATCACAC ATTTAGTGTG
3601	CTATTACCGG GATAATGGCC	CTGCAACTGG GACGTTGACC	TAGTTAAGAG ATCAATTCTC	AGCTGCAGCT TCGACGTCGA	GCCGTCATCC CGGCAGTAGG
3651	CTGAGCAGGG GACTCGTCCC	GGGCCACTTC CCCGGTGAAG	GTTAAGCATG CAATTCGTAC	TCCCTGACTC AGGGACTGAG	GCATGTTTTC CGTACAAAAG

Figure 27D

3701	CCTGACCAAA GGACTGGTTT	ECCAGAA AGGCGGTCTT	GGCGCTCGCC CCGCGAGCGG	GCCCAGCGAT CGGGTCGCTA	AGCAGT TT TCGTCAAGAA
3751				GACCGTCCGC CTGGCAGGCG	
3801	CTTTTGAGCG GAAAACTCGC	TTTGACCAAG AAACTGGTTC	CAGTTCCAGG GTCAAGGTCC	CGGTCCCACA GCCAGGGTGT	GCTCGGTCAC CGAGCCAGTG
3851				TCCTCGTTTC AGGAGCAAAG	
3901	CGCCGAAAGC	GACATGCCGT	CATCAGCCAC	CTCGTCCAGA GAGCAGGTCT	GCCCGGTCCC
3951	AGTACAGAAA	GGTGCCCGCG	TCCCAGGAGC	TCAGCGTAGT AGTCGCATCA	GACCCAGTGC
4001	CACTTCCCCA	CGCGAGGCCC	GACGCGCGAC	GCCAGGGTGC CGGTCCCACG	CGAACTCCGA
4051	CCAGGACGAC	CACGACTTCG	CGACGGCCAG	TTCGCCCTGC AAGCGGGACG	CGCAGCCGGT
4101	CCATCGTAAA	CTGGTACCAC	AGTATCAGGT	GCCCCTCCGC CGGGGAGGCG	CCGCACCGGG
4151	AACCGCGCGT	CGAACGGGAA	CCTCCTCCGC	CCGCACGAGG GGCGTGCTCC	CCGTCACGTC
4201	TGAAAACTCC	CGCATCTCGA	ACCCGCGCTC	AAATACCGAT TTTATGGCTA	AGGCCCCTCA
4251	TCCGTAGGCG	CGGCGTCCGG	GGCGTCTGCC	TCTCGCATTC AGAGCGTAAG	GTGCTCGGTC
4301	CACTCGAGAC	CGGCAAGCCC	CAGTTTTTGG	AGGTTTCCCC TCCAAAGGGG	GTACGAAAAA
4351	CTACGCAAAG	AATGGAGACC	AAAGGTACTC	CCGGTGTCCA GGCCACAGGT	GCGAGCCACT
4401	GCTTTTCCGA	CAGGCACAGG	GGCATATGTC	ACTTGAGAGG TGAACTCTCC	GGACAGGAGC
		GCGCCAGGAG	GAGCATATCT	TTGAGCCTGG	TGAGACTCTG
		CAGGTCCGGT	CGTGCTTCCT	CCGATTCACC	CTCCCCATCG
	CCAGCAACAG	GTGATCCCCC	AGGTGAGCGA	GGTCCCACAC	AAGACACATG TTCTGTGTAC
4601	TCGCCCTCTT AGCGGGAGAA	CGGCATCAAG GCCGTAGTTC	GAAGGTGATT CTTCCACTAA	GGTTTGTAGG CCAAACATCC	TGTAGGCCAC ACATCCGGTG

Figure 27E

4651	GTGACCGGGT CACTGGCCCA	CAAGGACTTC	GGGGGCTATA CCCCCGATAT	AAAGGGGGTG TTTCCCCCAC	GGGGC TT CCCCGCGCAA
4701				CGAGGGCCAG GCTCCCGGTC	
4751				TCTGCGCTAA AGACGCGATT	
4801			-	CTGGCCCGCG GACCGGGCGC	
4851				AGACAATCTT TCTGTTAGAA	
4901	TCGAACCACC	GTTTGCTGGG	CATCTCCCGC	TTGGACAGCA AACCTGTCGT	TGAACÇGCTA
4951	CCTCGCGTCC	CAAACCAAAA	ACAGCGCTAG	GGCGCGCTCC CCGCGCGAGG	AACCGGCGCT
5001 .	TGTTTAGCTG ACAAATCGAC			ACCGCCATTC TGGCGGTAAG	
5051				CGCCAACCGC GCGGTTGGCG	
5101				TCCGCGTAGG AGGCGCATCC	
5151	AGGTCGTCTC	CGCCGGCGGG	AACGCGCTCG	AGAATGGCGG TCTTACCGCC	ATCCCCCAGA
5201				ACGGTAAAGA TGCCATTTCT	
5251	GTCCGCGCGC	AGCTTCATCA	GATAGAACGT	TCCTTGCAAG AGGAACGTTC	AGATCGCGGA
5301				CGTATGGGTT GCATACCCAA	
5351	GGGGTACCGT	ACCCCACCCA	CTCGCGCCTC	GCGTACATGC CGCATGTACG	GCGTTTACAG
		TCCCCGAGAG	ACTCATAAGG	TTCTATACAT	CCCATCGTAG
5451	TTCCACCGCG AAGGTGGCGC	GATGCTGGCG CTACGACCGC	CGCACGTAAT GCGTGCATTA	CGTATAGTTC GCATATCAAG	GTGCGAGGGA CACGCTCCCT
		GCCCTGGCTC	CAACGATGCC	CGCCCGACGA	GACGAGCCTT
5551	GACTATCTGC CTGATAGACG			GGATGATATG CCTACTATAC	

Figure 27F

		c c c c c c c c c c c c c c c c c c c	momomes CBC	CTACCGCGTC	ACGCAGE G
5601	GGAAGACGTT	CTTCCCC	TCTGTGAGAC	GATGGCGCAG	TECETE
	CCTTCTGCAA	CTTCGACCGC	AGACACTCTG	GAIGGCGCAG	1000100110
	GAGGCGTAGG	> cmoccoco		» CCTCCCCCC	ጥር እርርጥርር እር
5651	GAGGCGTAGG	AGTCGCGCAG	CITGITGACC	TCGAGCCGCC	ACTECACETE
	CTCCGCATCC	TCAGCGCGTC	GAACAACIGG	TEGAGECEGEE	AC 1 GGACG 1 G
		a. a		GATGATGTCA	<u>ጥ</u> እርጥጥ አጥር ርጥ
5701				CTACTACAGT	
	CAGATCCCGC	GTCATCAGGT	CCCAAAGGAA	CIACIACAGI	MICANIADIA
		mmmaa.a.a.a.	maccacamac N	GGACAAACTC	MACCCCCATCT
5751	GTCCCTTTTT	TTTCCACAGC	TUGUGGTTGA	CCTGTTTGAG	AACCCCAGA
	CAGGGAAAAA	AAAGGIGICG	AGCGCCAACT	CCIGITIGAG	AAGCGCCAGA
			>>> CCCCCTCC	GCCTCCGAAC	CCTANGAGCC
5801	TTCCAGTACT	CTTGGATCGG	AAACCCGICG	CCCACCCTTC	CCATTCTCCC
	AAGGTCATGA	GAACCTAGCC	TTTGGGCAGC	CGGAGGCTTG	CCATTCTCGG
		**********	account of a	GGCGCAGCAT	CCCጥጥጥጥCጥA
5851	TAGCATGTAG	AACTGGTTGA	CGGCCTGGTA	CCGCGTCGTA	CCCITICIA
	ATCGTACATC	TIGACCAACT	GUUGGACUAT	CCGCGICGIA	GGGWWGWI
	000000000000000000000000000000000000000	CM > IDCCCIMCC	CCCCCTTCC	GGAGCGAGGT	CTCCCTCACC
5901	CGGGTAGCGC	GIAIGCCIGC	GCGGCCTTCC	CCTCGCTCCA	CACCCACTCG
	GCCCATCGCG	CATACGGACG	CGCCGGAAAGG	CCICGCICCA	Checonordo
		CCCCCACCAM	. כארששיניאנים	TACTGGTATT	TGAAGTCAGT
5951	GCAAAGGTGT	CCCTGACCAT	GACIIIGAGG	ATGACCATAA	ACTTCAGTCA
	CGTTTCCACA	GGGACTGGTA	CIGAMACICC	AIGACCAIAA	ACTICACTOR
5000	omoomoogam	COCCCCCCCC	CCCACACCAA	AAAGTCCGTG	СССТТТТТСС
6001	GTCGTCGCAT	CCGCCCTGCT	CCCAGAGCAA	TTTCAGGCAC	CCCYYYYYCC
	CAGCAGCGTA	GGCGGGACGA	GGGTCTCGTT	IIICAGGCAC	GCGHBBB 100
COE1	**************************************	TO CONTOCO	БЪССТСЪСЪТ	CGTTGAAGAG	TATCTTTCCC
6051	MACGCGGA11	ACCOMOCCC	TOTOCACTO	GCAACTTCTC	ATAGAAAGGG
	TIGUGUCIAA	ACCGICCCGC	TICCHCIGIA	00.2.0.0	
6101	CCCCCACCCA	ጥልልልርጥጥርርር	TOTEATECES	AAGGGTCCCG	GCACCTCGGA
9101	CCCCCTCCCT	משתדר ממרכר	ACACTACGCC	TTCCCAGGGC	CGTGGAGCCT
	CGCGCTCCGT	ATTICIANCOC	7.0	,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,	
6151	ACCOTTOTTA	ልጥጥልCCጥርርር	CGGCGAGCAC	GATCTCGTCA	AAGCCGTTGA
0131	TOCCARCAR	TAATGCACCC	GCCGCTCGTG	CTAGAGCAGT	TTCGGCAACT
	IGCCAACAAT	IMIGGACCC	500001011	•	
6201	ጥርጥጥርጥርርር	САСААТСТАА	AGTTCCAAGA	AGCGCGGGAT	GCCCTTGATG
0201	ACAACACCGG	GTGTTACATT	TCAAGGTTCT	TCGCGCCCTA	CGGGAACTAC
	ACAMENCEOU	0.011			
6251	GAAGGCAATT	TTTTAAGTTC	CTCGTAGGTG	AGCTCTTCAG	GGGAGCTGAG
0232	CTTCCGTTAA	AAAATTCAAG	GAGCATCCAC	TCGAGAAGTC	CCCTCGACTC
	000000000				
6301	CCCGTGCTCT	GAAAGGGCCC	AGTCTGCAAG	ATGAGGGTTG	GAAGCGACGA
0000	GGGCACGAGA	CTTTCCCGGG	TCAGACGTTC	TACTCCCAAC	CTTCGCTGCT
		•			
6351	ATGAGCTCCA	CAGGTCACGG	GCCATTAGCA	TTTGCAGGTG	GTCGCGAAAG
	TACTCGAGGT	GTCCAGTGCC	CGGTAATCGT	AAACGTCCAC	CAGCGCTTTC
6401	GTCCTAAACT	GGCGACCTAT	GGCCATTTTT	TCTGGGGTGA	TGCAGTAGAA
	CAGGATTTGA	CCGCTGGATA	CCGGTAAAAA	AGACCCCACT	ACGTCATCTT
6451	GGTAAGCGGG	TCTTGTTCCC	AGCGGTCCCA	TCCAAGGTTC	GCGGCTAGGT
	CCATTCGCCC	AGAACAAGGG	TCGCCAGGGT	AGGTTCCAAG	CGCCGATCCA
6501	CTCGCGCGGC	AGTCACTAGA	GGCTCATCTC	CGCCGAACTT	CATGACCAGC
	GAGCGCGCCG	TCAGTGATCT	CCGAGTAGAG	GCGGCTTGAA	GTACTGGTCG

Figure 27G

6551		CTCGACGAA			TATACO CO ATATCCAGAG
6601		GTGACAAAGA CACTGTTTCT			
6651	•	GATCTCCCGC CTAGAGGGCG			
6701		AGTCCCTGCG TCAGGGACGC			
6751		CAGTACTGGC GTCATGACCG			
6801		ACGACCGCGC TGCTGGCGCG			
6851	TCGCCTGGCG	GGTTTGGCTG CCAAACCGAC	GTGGTCTTCT	ACTTCGGCTG	CTTGTCCTTG
6901	ACCGTCTGGC	TGCTCGAGGG ACGAGCTCCC	GAGTTACGGT	GGATCGGACC	ACCACGCCGC
6951	GCGAGCCCAA	AGTCCAGATG TCAGGTCTAC	TCCGCGCGCG	GCGGTCGGAG	CTTGATGACA
7001	ACATCGCGCA	GATGGGAGCT CTACCCTCGA	GTCCATGGTC	TGGAGCTCCC	GCGGCGTCAG
7051	GTCAGGCGGG	AGCTCCTGCA TCGAGGACGT	GGTTTACCTC	GCATAGACGG	GTCAGGGCGC
7101	GGGCTAGATC	CAGGTGATAC	СТААТТТССА	GGGGCTGGTT	GGTGGCGGCG
7151	TCGATGGCTT	GCAAGAGGCC	GCATCCCCGC	GGCGCGACTA	CGGTACCGCG
7201	CGCCGGCGG	TGGCCGCGG	GGGTĠTCCTT	GGATGATGCA	TCTAAAAGCG
7251	GTGACGCGGG	CCACCCCCC	GAGGTAGGGG	GGGCTCCGGA	CCCGCCGGGA
7301		GCTCGGGGGC GGGCACGTCG			
7351		TGCTGGCGAA			
7401		ACGACCGCTT TGCGTGAAGA			
7451		ACGCACTTCT AGAATCAATT			
		TCTTAGTTAA			

Figure 27H

7501	ATCTCCTGCA TAGAGGACGT	CACCTCCTGA	GTTGTCTTGA CAACAGAACT	TAGGCGATUL ATCCGCTAGA	GCCGGT TT
7551	CTGCTCGATC	TCTTCCTCCT	GGAGATCTCC	GCGTCCGGCT	CGCTCCACGG
7601			CCTCTAGAGG ATGCGGGCCA		
	ACCGCCGCTC	CAGCAACCTT	TACGCCCGGT	ACTCGACGCT	CTTCCGCAAC
7651			GCGGCTGTAG CGCCGACATC		
7701			GCGCGAGATT CGCGCTCTAA		
7751	AGACGGCGTA TCTGCCGCAT	GTTTCGCAGG CAAAGCGTCC	CGCTGAAAGA GCGACTTTCT	GGTAGTTGAG CCATCAACTC	GGTGGTGGCG CCACCACCGC
7801	GTGTGTTCTG CACACAAGAC	CCACGAAGAA GGTGCTTCTT	GTACATAACC CATGTATTGG	CAGCGTCGCA GTCGCAGCGT	ACGTGGATTC TGCACCTAAG
7851			CAAGGCGCTC GTTCCGCGAG		
7901	CGGCGAAGTT GCCGCTTCAA	GAAAAACTGG CTTTTTGACC	GAGTTGCGCG CTCAACGCGC	CCGACACGGT GGCTGTGCCA	TAACTCCTCC ATTGAGGAGG
7951			GGCGACAGTG CCGCTGTCAC		
8001			CTTCTTCAAT GAAGAAGTTA		
8051			GCCGCCACCCC		
8101			GTCGACAAAG CAGCTGTTTC		
8151			TGACGGCGCG ACTGCCGCGC		
8201			ATGTCCCGGT TACAGGGCCA		
8251	CCATGCGGCA GGTACGCCGT	GGGATACGGC CCCTATGCCG	GCTAACGATG CGATTGCTAC	CATCTCAACA GTAGAGTTGT	ATTGTTGTGT TAACAACACA
8301	AGGTACTCCG TCCATGAGGC	CCGCCGAGGG GGCGCTCCC	ACCTGAGCGA TGGACTCGCT	GTCCGCATCG CAGGCGTAGC	ACCGGATCGG TGGCCTAGCC
8351	AAAACCTCTC TTTTGGAGAG	GAGAAAGGCG CTCTTTCCGC	TCTAACCAGT AGATTGGTCA	CACAGTCGCA GTGTCAGCGT	AGGTAGGCTG TCCATCCGAC
8401	AGCACCGTGG TCGTGGCACC	CGGGCGGCAG GCCCGCCGTC	CGGCGGCGG	TCGGGGTTGT AGCCCCAACA	TTCTGGCGGA AAGACCGCCT

Figure 27I

8451			CCCCCTACC
8501		 TTGGGTCCGG AACCCAGGCC	GCGCAGGCGG CGCGTCCGCC
8551		GTTTTGACAT CAAAACTGTA	
8601		 CCGGCACTTC GGCCGTGAAG	
8651		GCTGCGGCGG CGACGCCGCC	
8701		 GCGTGTGACC CGCACACTGG	
8751		CAACGCGCTC GTTGCGCGAG	
8801		AAGTCATCCA TTCAGTAGGT	
8851		 AGTGCAGTTG TCACGTCAAC	
8901		AGAGCTCGGT TCTCGAGCCA	
8951		TCGTTGCAAG AGCAACGTTC	
9001		CGGCTGGCGG GCCGACCGCC	
9051		GATCTTCCAA CTAGAAGGTT	
9101		GTGATGCCGG CACTACGGCC	
9151		CCAGATGTTG GGTCTACAAC	
9201	CATGGTCGGG GTACCAGCCC	CGGTCAGGCG GCCAGTCCGC	
9251	AGACCGTGCA TCTGGCACGT	CTGTAAGCGG GACATTCGCC	
9301	GGATAAATTC CCTATTTAAG	CATGGCGGAC GTACCGCCTG	
9351	ATCCGGCCGT TAGGCCGGCA	CCATGCGGTT GGTACGCCAA	

Figure 27J

9401	AGGTGTGCGA	O AGACAA	CGGGGGAGTG	CTCCTTTTGG	CTTCCT
	•	GCAGTCTGTT		•	
9451	GCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC	CTGCTGCGCT GACGACGCGA	AGCTTTTTTG TCGAAAAAAC	GCCACTGGCC	GCGCGCAGCG CGCGCGTCGC
0501	TAAGCGGTTA				
3201		CCGACCTTTC			
9551	CCGGAGGGTT	ATTTTCCAAG	GGTTGAGTCG	CGGGACCCCC	GGTTCGAGTC
•	GGCCTCCCAA	TAAAAGGTTC	CCAACTCAGC	GCCCTGGGGG	CCAAGCTCAG
9601	TCGGACCGGC				
	AGCCTGGCCG	GCCTGACGCC	GCTTGCCCCC	AAACGGAGGG	GCAGTACGTT
9651	GACCCCGCTT	GCAAATTCCT	CCGGAAACAG	GGACGAGCCC	CTTTTTTGCT
	CTGGGGCGAA	CGTTTAAGGA	GGCCTTTGTC	CCTGCTCGGG	GAAAAAACGA
9701	TTTCCCAGAT				
	AAAGGGTCTA	CGTAGGCCAC	GACGCCGTCT	ACGCGGGGGG	AGGAGTCGTC
9751		AAGAGCAGCG			
	GCCGTTCTCG	TTCTCGTCGC	CGTCTGTACG	TCCCGTGGGA	GGGGAGGAGG
9801	TACCGCGTCA	GGAGGGGCGA	CATCCGCGCT	TGACGCGGCA	GCAGATGGTG
	ATGGCGCAGT	CCTCCCCGCT	GTAGGCGCCA	ACTGCGCCGT	CGTCTACCAC
9851	ATTACGAACC	CCCGCGGCGC	CGGCCCGGC	ACTACCTGGA	CTTGGAGGAG
		GGCGCCGCG			
9901	GGCGAGGGCC	TGGCGCGGCT	AGGAGCGCCC	TCTCCTGAGC	GCCACCCAAG
		ACCGCGCCGA			
9951	GGTGCAGCTG	AAGCGTGATA	CGCGTGAGGC	GTACGTGCCG	CGGCAGAACC
	CCACGTCGAC	TTCGCACTAT	GCGCACTCCG	CATGCACGGC	GCCGTCTTGG
10001	TGTTTCGCGA	CCGCGAGGGA	GAGGAGCCCG	AGGAGATGCG	GGATCGAAAG
		GGCGCTCCCT			
10051	TTCCACGCAG	GGCGCGAGCT	GCGGCATGGC	CTGAATCGCG	AGCGGTTGCT
	AAGGTGCGTC	CCGCGCTCGA	CGCCGTACCG	GACTTAGCGC	TCGCCAACGA
10101	GCGCGAGGAG	GACTTTGAGC	CCGACGCGCG	AACCGGGATT	AGTCCCGCGC
	CGCGCTCCTC	CTGAAACTCG	GGCTGCGCGC	TTGGCCCTAA	TCAGGGCGCG
10151	GCGCACACGT	GGCGGCCGCC	GACCTGGTAA	CCGCATACGA	GCAGACGGTG
	CGCGTGTGCA	CCGCCGGCGG	CTGGACCATT	GGCGTATGCT	CGTCTGCCAC
10201	AACCAGGAGA	TTAACTTTCA	AAAAAGCTTT	AACAACCACG	TGCGTACGCT
	TTGGTCCTCT	AATTGAAAGT	TTTTTCGAAA	TTGTTGGTGC	ACGCATGCGA
10251	TGTGGCGCGC	GAGGAGGTGG	CTATAGGACT	GATGCATCTG	TGGGACTTTG
	ACACCGCGCG	CTCCTCCACC	GATATCCTGA	CTACGTAGAC	ACCCTGAAAC
10301	TAAGCGCGCT	GGAGCAAAAC	CCAAATAGCA	AGCCGCTCAT	GGCGCAGCTG
	ATTCGCGCGA	CCTCGTTTTG	GGTTTATCGT	TCGGCGAGTA	CCGCGTCGAC

Figure 27K

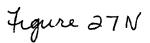
10351					GGGATG T CCCTACG-GA
10401					TTGATAAACA AACTATTTGT
10451		CATAGTGGTG GTATCACCAC			GGCTGACAAG CCGACTGTTC
10501		TCAACTATTC AGTTGATAAG			TTTACGCCCG AAATGCGGGC
10551	_	CATACCCCTT GTATGGGGAA			
10601		CATGCGCATG GTACGCGTAC			
10651		ATCGCAACGA TAGCGTTGCT			
10701		CTCAGCGACC GAGTCGCTGG			
10751		GGGCAGCGGC CCCGTCGCCG			
10801		TGCGCTGGGC ACGCGACCCG			
10851		GGGCTGGCGG CCCGACCGCC			
10901		ATATGACGAG TATACTGCTC			
10951		TGATGTTTCT ACTACAAAGA			
11001		CGGCGCTGCA GCCGCGACGT			
11051	. CGACTGGCGC GCTGACCGCG	CAGGTCATGG GTCCAGTACC			
11101	CTGACGCGTT GACTGCGCAA	CCGGCAGCAG GGCCGTCGTC			
11151	GAAGCGGTGG CTTCGCCACC	TCCCGGCGCGC AGGGCCGCGC			
11201	GATCGTAAAC CTAGCATTTG	GCGCTGGCCG CGCGACCGGC	AAAACAGGGC TTTTGTCCCG	CATCCGGCCC GTAGGCCGGG	GACGAGGCCG CTGCTCCGGC
11251	GCCTGGTCTA CGGACCAGAT				CAACAGCGGC GTTGTCGCCG

Figure 27L

11301	AACGTGCAGA TTGCACGTCT	CCTGGA GGTTGGACCT	CCGGCTGGTG GGCCGACCAC	CCCCTACACG	GCGAGG T CGCTCCGGCA
11351	GGCGCAGCGT	GAGCGCGCGC	AGCAGCAGGG	CAACCTGGGC	TCCATGGTTG
	CCGCGTCGCA	CTCGCGCGCG	TCGTCGTCCC	GTTGGACCCG	AGGTACCAAC
11401	CACTAAACGC	CTTCCTGAGT	ACACAGCCCG	CCAACGTGCC	GCGGGGACAG
	GTGATTTGCG	GAAGGACTCA	TGTGTCGGGC	GGTTGCACGG	CGCCCTGTC
11451	GAGGACTACA	CCAACTTTGT	GAGCGCACTG	CGGCTAATGG	TGACTGAGAC
	CTCCTGATGT	GGTTGAAACA	CTCGCGTGAC	GCCGATTACC	ACTGACTCTG
11501	ACCGCAAAGT	GAGGTGTACC	AGTCTGGGCC	AGACTATTTT	TTCCAGACCA
	TGGCGTTTCA	CTCCACATGG	TCAGACCCGG	TCTGATAAAA	AAGGTCTGGT
11551	GTAGACAAGG	CCTGCAGACC	GTAAACCTGA	GCCAGGCTTT	CAAAAACTTG
	CATCTGTTCC	GGACGTCTGG	CATTTGGACT	CGGTCCGAAA	GTTTTTGAAC
11601	CAGGGGCTGT GTCCCCGACA	GGGGGGTGCG CCCCCACGC	GGCTCCCACA CCGAGGGTGT	CCCCTGCCG	CGACCGTGTC GCTGGCACAG
11651	ATCGAACGAC	TGCGGGTTGA	GCGCGGACAA	GCTGCTGCTA CGACGACGAT	TATCGCGGGA
11701	TCACGGACAG	TGGCAGCGTG	TCCCGGGACA	CATACCTAGG	TCACTTGCTG
	AGTGCCTGTC	ACCGTCGCAC	AGGGCCCTGT	GTATGGATCC	AGTGAACGAC
11751	ACACTGTACC	GCGAGGCCAT	AGGTCAGGCG	CATGTGGACG	AGCATACTTT
	TGTGACATGG	CGCTCCGGTA	TCCAGTCCGC	GTACACCTGC	TCGTATGAAA
11801	CCAGGAGATT GGTCCTCTAA	ACAAGTGTCA TGTTCACAGT	GCCGCGCGCT CGGCGCGCGA	GGGGCAGGAG	GACACGGGCA CTGTGCCCGT
11851	GCCTGGAGGC	AACCCTAAAC TTGGGATTTG	TACCTGCTGA ATGGACGACT	CCAACCGGCG GGTTGGCCGC	GCAGAAGATC CGTCTTCTAG
11901	CCCTCGTTGC	ACAGTTTAAA	CAGCGAGGAG	GAGCGCATTT	TGCGCTACGT
	GGGAGCAACG	TGTCAAATTT	GTCGCTCCTC	CTCGCGTAAA	ACGCGATGCA
11951	GCAGCAGAGC	GTGAGCCTTA	ACCTGATGCG	CGACGGGGTA	ACGCCCAGCG
	CGTCGTCTCG	CACTCGGAAT	TGGACTACGC	GCTGCCCCAT	TGCGGGTCGC
12001	TGGCGCTGGA	CATGACCGCG	CGCAACATGG	AACCGGGCAT	GTATGCCTCA
	ACCGCGACCT	GTACTGGCGC	GCGTTGTACC	TTGGCCCGTA	CATACGGAGT
12051	AACCGGCCGT TTGGCCGGCA	TTATCAACCG AATAGTTGGC	CCTAATGGAC GGATTACCTG	TACTTGCATC ATGAACGTAG	CGCGCCGCC
12101	CGTGAACCCC GCACTTGGGG	GAGTATTTCA CTCATAAAGT	CCAATGCCAT GGTTACGGTA	CTTGAACCCG GAACTTGGGC	CACTGGCTAC
12151	CGCCCCTGG	TTTCTACACC AAAGATGTGG	GGGGGATTCG CCCCTAAGC	AGGTGCCCGA TCCACGGGCT	GGGTAACGAT
12201	GGATTCCTCT	GGGACGACAT	AGACGACAGO	GTGTTTTCCC	CGCAACCGCA
	CCTAAGGAGA	CCCTGCTGTA	TCTGCTGTCG	CACAAAAGGG	GCGTTGGCGT

Figure 27 M

12251					CCCCTC
	CTGGGACGAT	CAACGTTG	TCGCGCTCGT	CCGTCTCCGC	CGCGAC
12301					CGCTGCGGCC
	TCCTTTCGAA	GGCGTCCGGT	TCGTCGAACA	GGCTAGATCC	GCGACGCCGG
12351					GGTCTCTTAC
	GGCGCCAGIC	TACGATCATC	GGGTAAAGGT	TCGAACTATC	CCAGAGAATG
12401					GAGTACCTAA CTCATGGATT
	GTCGTGAGCG	106166666	GUGUGGAUGA	CCCGCTCCTC	CTCATGGATT
12451					TCCGGCATTT
	TGTTGAGCGA	CGACGTCGGC	GTCGCGCTTT	TTTTGGACGG	AGGCCGTAAA
12501		GGATAGAGAG			
	GGGTTGTTGC	CCTATCTCTC	GGATCACCTG	TTCTACTCAT	CTACCTTCTG
12551					CCCACCCGTC
	CATGCGCGTC	CTCGTGTCCC	TGCACGGTCC	GGGCGCGGGC	GGGTGGGCAG
12601	GTCAAAGGCA	CGACCGTCAG	CGGGGTCTGG	TGTGGGAGGA	CGATGACTCG
	CAGTTTCCGT	GCTGGCAGTC	GCCCCAGACC	ACACCCTCCT	GCTACTGAGC
12651	GCAGACGACA	GCAGCGTCCT	GGATTTGGGA	GGGAGTGGCA	ACCCGTTTGC
	CGTCTGCTGT	CGTCGCAGGA	CCTAAACCCT	CCCTCACCGT	TGGGCAAACG
12701	GCACCTTCGC	CCCAGGCTGG	GGAGAATGTT	TTAAAAAAAA	AAAAAGCATG
	CGTGGAAGCG	GGGTCCGACC	CCTCTTACAA	AATTTTTTTT	TTTTTCGTAC
12751	ATGCAAAATA	AAAAACTCAC	CAAGGCCATG	GCACCGAGCG	TTGGTTTTCT
	TACGTTTTAT	TITTTGAGTG	GTTCCGGTAC	CGTGGCTCGC	AACCAAAAGA
12801		TIAGTATGCG			
	ACATAAGGGG	AATCATACGC	CGCGCGCCGC	TACATACTCC	TTCCAGGAGG
12851	TCCCTCCTAC	GAGAGTGTGG	TGAGCGCGGC	GCCAGTGGCG	GCGGCGCTGG
	AGGGAGGATG	CICTCACACC	ACTCGCGCCG	CGGTCACCGC	CGCCGCGACC
12901		CGATGCTCCC			
	CAAGAGGGAA	GCTACGAGGG	GACCTGGGCG	GCAAACACGG	AGGCGCCATG
12951	CTGCGGCCTA	CCGGGGGGAG	AAACAGCATC	CGTTACTCTG	AGTTGGCACC
	GACGCCGGAT	GGCCCCCTC	TTTGTCGTAG	GCAATGAGAC	TCAACCGTGG
13001	CCTATTCGAC	ACCACCCGTG	TGTACCTGGT	GGACAACAAG	TCAACGGATG
	GGATAAGCTG	TGGTGGGCAC	ACATGGACCA	CCTGTTGTTC	AGTTGCCTAC
13051	TGGCATCCCT	GAACTACCAG	AACGACCACA	GCAACTTTCT	GACCACGGTC
	ACCGTAGGGA	CTTGATGGTC	TTGCTGGTGT	CGTTGAAAGA	CTGGTGCCAG
13101					AGACCATCAA
	TAAGTTTTGT	TACTGATGTC	GGGCCCCCTC	CGTTCGTGTG	TCTGGTAGTT
13151	TCTTGACGAC	CGGTCGCACT	GGGGCGGCGA	CCTGAAAACC	ATCCTGCATA
	AGAACTGCTG	GCCAGCGTGA	CCCCCCCCT	GGACTTTTGG	TAGGACGTAT



			GAGTTCATGT	mmaccaamaa	ማውው ይመው መጀመር ነው። የመደር መስመር ነው የመጀመር ነው
13201	CCAACATGCC	AA GIGAAC	CTCAAGTACA	AAMCCMAIAA	CALANTA
	GGTTGTACGG	TE ACACTIC	CTCAAGTACA	WIGGIIVII	Christia
		MOMO COCOM	GCCTACTAAG	CACAATCAGG	ጥርርእርርጥርእእ
13251	CGGGTGATGG	1616666611	CGGATGATTC	CHCHATCAGG	ACCTCCACTT
	GCCCACTACC	ACAGCGCGAA	CGGATGATTC	CIGITAGICC	Accicancii
	> m > 00 > 0 m 0 0		CGCTGCCCGA	CCCCAACTAC	TOTOLOGICA
13301	ATACGAGTGG	CACCECARTICA	GCGACGGGCT	CCCCTTCATC	ACCCTCTCCT
	TATGCTCACC	CACCICAAGI	GCGACGGGCI	CCCGIIGAIG	A000101001
13351	TC A C C A TA C A	ССФТАТСААС	AACGCGATCG	TEGAGCACTA	CTTGAAAGTG
13331	ACTICCTATION	CCAATACTTG	TTGCGCTAGC	ACCTCGTGAT	GAACTTTCAC
	ACIGGIAICI				
13401	GGCAGACAGA	ACGGGGTTCT	GGAAAGCGAC	ATCGGGGTAA	AGTTTGACAC
13401	CCGTCTGTCT	TGCCCCAAGA	CCTTTCGCTG	TAGCCCCATT	TCAAACTGTG
	cc0101010;				
13451	CCGCAACTTC	AGACTGGGGT	TTGACCCCGT	CACTGGTCTT	GTCATGCCTG
13431	GGCGTTGAAG	TCTGACCCCA	AACTGGGGCA	GTGACCAGAA	CAGTACGGAC
13501	GGGTATATAC	AAACGAAGCC	TTCCATCCAG	ACATCATTTT	GCTGCCAGGA
	CCCATATATG	TTTGCTTCGG	AAGGTAGGTC	TGTAGTAAAA	CGACGGTCCT
13551	TGCGGGGTGG	ACTTCACCCA	CAGCCGCCTG	AGCAACTTGT	TGGGCATCCG
	ACGCCCCACC	TGAAGTGGGT	GTCGGCGGAC	TCGTTGAACA	ACCCGTAGGC
13601	CAAGCGGCAA	CCCTTCCAGG	AGGGCTTTAG	GATCACCTAC	GATGATCTGG
	GTTCGCCGTT	GGGAAGGTCC	TCCCGAAATC	CTAGTGGATG	CTACTAGACC
13651	AGGGTGGTAA	CATTCCCGCA	CTGTTGGATG	TGGACGCCTA	CCAGGCGAGC
	TCCCACCATT	GTAAGGGCGT	GACAACCTAC	ACCTGCGGAT	GGTCCGCTCG
				0000000000	CCACCAACAC
13701	TTGAAAGATG	ACACCGAACA	GGGCGGGGGT	GGCGCAGGCG	CCAGCAACAG
	AACTTTCTAC	TGTGGCTTGT	CCCGCCCCCA	CCGCGTCCGC	6616611616
		000000000000000000000000000000000000000	AGAACTCCAA	CCCCCCACCC	CCCCCAATCC
13751	CAGTGGCAGC	GGCGCGGAAG	TCTTGAGGTT	CCCCCCTCCC	CCCCCTTACC
	GTCACCGTCG	CCGCGCCTIC	ICIIGAGGII	6060001000	20000111100
13801	ACCCCCTCCA	CCACATGAAC	GATCATGCCA	TTCGCGGCGA	CACCTTTGCC
13801	MCCCCC3 CCT	CCTCTACTTC	CTAGTACGGT	AAGCGCCGCT	GTGGAAACGG
	ICGGCCACCI	CCIGIACIIG	Cindingooi		0100 ,11111
13851	ACACGGGCTG	ACCAGAAGCG	CGCTGAGGCC	GAAGCAGCGG	CCGAAGCTGC
13031	TCTCCCCGAC	TCCTCTTCGC	GCGACTCCGG	CTTCGTCGCC	GGCTTCGACG
	.01000000		• • • • • • • • • • • • • • • • • • • •		
13901	CGCCCCCGCT	GCGCAACCCG	AGGTCGAGAA	GCCTCAGAAG	AAACCGGTGA
10,00	GCGGGGGGGA	CGCGTTGGGC	TCCAGCTCTT	CGGAGTCTTC	TTTGGCCACT
13951	TCAAACCCCT	GACAGAGGAC	AGCAAGAAAC	GCAGTTACAA	CCTAATAAGC
	AGTTTGGGGA	CTGTCTCCTG	TCGTTCTTTG	CGTCAATGTT	GGATTATTCG
14001	AATGACAGCA	CCTTCACCCA	GTACCGCAGC	TGGTACCTTG	CATACAACTA
	TTACTGTCGT	GGAAGTGGGT	CATGGCGTCG	ACCATGGAAC	GTATGTTGAT
14051	CGGCGACCCT	CAGACCGGAA	TCCGCTCATG	GACCCTGCTT	TGCACTCCTG
	GCCGCTGGGA	GTCTGGCCTT	AGGCGAGTAC	CTGGGACGAA	ACGIGAGGAC
				000000000	>C>C>C>CC
14101	ACGTAACCTG	CGGCTCGGAG	CAGGTCTACT	GGTCGTTGCC	AGACATGATG
	TGCATTGGAC	GCCGAGCCTC	GICCAGATGA	CLAGCAACGG	TCTGTACTAC

Tigure 270

14151	CAAGACCCCG				ACTTTC
14201		CAGCTGTTGC			
14201		CTCGACAACG			
14251		CTCCCAACTC GAGGGTTGAG			
14301		TTCCCGAGAA			
		AAGGGCTCTT			
14351		GTCAGTGAAA CAGTCACTTT			
14401		CAACAGCATC GTTGTCGTAG			
14451		GCACCTGCCC			
	CGGTCTGCGG	CGTGGACGGG	GATGCAAATG	TTCCGGGACC	CGTATCAGAG
14501		CTATCGAGCC GATAGCTCGG			
14551		CAATAACACA			
	ATAGCGGGTC	GTTATTGTGT	CCGACCCCGG	ACGCGAAGGG	TTCGTTCTAC
14601		CCAAGAAGCG			
	AAACCGCCCC	GGTTCTTCGC	GAGGCTGGTT	GTGGGTCACG	CGCACGCGCC
14651		GCGCCCTGGG			
	CGTGATGGCG	CGCGGGACCC	CGCGCGTGTT	TGCGCCGGCG	TGACCCGCGT
14701	•••••	TGACGCCATC	•		
	GGTGGCAGCT	ACTGCGGTAG	CTGCGCCACC	ACCTCCTCCG	CGCGTTGATG
14751	ACGCCCACGC	CGCCACCAGT	GTCCACAGTG	GACGCGGCCA	TTCAGACCGT
	TGCGGGTGCG	GCGGTGGTCA	CAGGTGTCAC	CTGCGCCGGT	AAGTCTGGCA
14801		GCCCGGCGCT			
	CCACGCCCCT	CGGGCCGCGA	TACGATTTTA	CTTCTCTGCC	GCCTCCGCGC
14851		CCACCGCCGC GGTGGCGGCG			
14901	GCGGCCCTGC	TTAACCGCGC	ACGTCGCACC	GGCCGACGGG	CGGCCATGCG
	CGCCGGGACG	AATTGGCGCG	TGCAGCGTGG	CCGGCTGCCC	GCCGGTACGC
14951	GCCCGCTCGA CCGGCGAGCT	AGGCTGGCCG TCCGACCGGC			
15001	GGCGACGAGC	GGCCGCCGCA	GCAGCCGCGG	CCATTAGTGC	TATGACTCAG
		CCGGCGGCGT			
15051	GGTCGCAGGG				
	CCAGCGTCCC	CGTTGCACAT	AACCCACGCG	CTGAGCCAAT	CGCCGGACGC

Figure 27P

15101	CGTGCCCGTG GCACGGGCAC	650000000000000000000000000000000000000	CCCCGCGCAA GGGGCGCGTT	CTAGATTGCA GATCTAACGT	TCTTTTT.A
15151	ACTTAGACTC TGAATCTGAG	GTACTGTTGT CATGACAACA	ATGTATCCAG TACATAGGTC	GCGCGGCGGC	GCGCAACGAA CGCGTTGCTT
15201	GCTATGTCCA	ACCGCAAAAT	CAAAGAAGAG	ATGCTCCAGG	TCATCGCGCC
	CGATACAGGT	TCGCGTTTTA	GTTTCTTCTC	TACGAGGTCC	AGTAGCGCGG
15251	GGAGATCTAT	GGCCCCCGA	AGAAGGAAGA	GCAGGATTAC	AAGCCCCGAA
	CCTCTAGATA	CCGGGGGGCT	TCTTCCTTCT	CGTCCTAATG	TTCGGGGCTT
15301	AGCTAAAGCG	GGTCAAAAAG	AAAAAGAAAG	ATGATGATGA	TGAACTTGAC
	TCGATTTCGC	CCAGTTTTTC	TTTTTCTCTC	TACTACTACT	ACTTGAACTG
15351	GACGAGGTGG	AACTGCTGCA	CGCTACCGCG	CCCAGGCGAC	GGGTACAGTG
	CTGCTCCACC	TTGACGACGT	GCGATGGCGC	GGGTCCGCTG	CCCATGTCAC
15401	GAAAGGTCGA	CGCGTAAAAC	GTGTTTTGCG	ACCCGGCACC	ACCGTAGTCT
	CTTTCCAGCT	GCGCATTTTG	CACAAAACGC	TGGGCCGTGG	TGGCATCAGA
15451	TTACGCCCGG	TGAGCGCTCC	ACCCGCACCT	ACAAGCGCGT	GTATGATGAG
	AATGCGGGCC	ACTCGCGAGG	TGGGCGTGGA	TGTTCGCGCA	CATACTACTC
15501	GTGTACGGCG	ACGAGGACCT	GCTTGAGCAG	GCCAACGAGC	GCCTCGGGGA
	CACATGCCGC	TGCTCCTGGA	CGAACTCGTC	CGGTTGCTCG	CCGAGCCCCT
15551	GTTTGCCTAC CAAACGGATG	CCTTTCGCCG	ATAAGGACAT TATTCCTGTA	GCTGGCGTTG CGACCGCAAC	CCGCTGGACG
15601	AGGGCAACCC	AACACCTAGC	CTAAAGCCCG	TAACACTGCA	GCAGGTGCTG
	TCCCGTTGGG	TTGTGGATCG	GATTTCGGGC	ATTGTGACGT	CGTCCACGAC
15651	CCCGCGCTTG	CACCGTCCGA	AGAAAAGCGC	GGCCTAAAGC	GCGAGTCTGG
	GGGCGCGAAC	GTGGCAGGCT	TCTTTTCGCG	CCGGATTTCG	CGCTCAGACC
15701	TGACTTGGCA	CCCACCGTGC	AGCTGATGGT	ACCCAAGCGC	CAGCGACTGG
	ACTGAACCGT	GGGTGGCACG	TCGACTACCA	TGGGTTCGCG	GICGCTGACC
15751	AAGATGTCTT	GGAAAAAATG	ACCGTGGAAC	CTGGGCTGGA	GCCCGAGGTC
	TTCTACAGAA	CCTTTTTTAC	TGGCACCTTG	GACCCGACCT	CGGGCTCCAG
15801	CCCCTCCGC	CAATCAAGCA GTTAGTTCGT	GGTGGCGCCG	GGACTGGGCG CCTGACCCGC	TGCAGACCGT ACGTCTGGCA
15851	GGACGTTCAG	ATACCCACTA	CCAGTAGCAC	CAGTATTGCC	ACCGCCACAG
	CCTGCAAGTC	TATGGGTGAT	GGTCATCGTG	GTCATAACGG	TGGCGGTGTC
15901	AGGGCATGGA TCCCGTACCT	GACACAAACG CTGTGTTTGC	TCCCCCCTTC AGGGGCCAAC	CCTCAGCGGT GGAGTCGCCA	CCCCCTACGG
15951	GCGGTGCAGG CGCCACGTCC	CGGTCGCTGC GCCAGCGACG	GGCCGCGTCC	AAGACCTCTA .TTCTGGAGAT	CGGAGGTGCA GCCTCCACGT
16001	AACGGACCCG TTGCCTGGGC	TGGATGTTTC ACCTACAAAG	GCGTTTCAGC CGCAAAGTCG	GGGGGCGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGG	CCGCGCCGTT GGCGCGGCAA

Figure 270

16051				TGCCCGAATA ACGGGCTTAT	
16101				GGCTACACCT CCGATGTGGA	
16151				CACTGGAACC GTGACCTTGG	
16201				TTTCCGTGCG AAAGGCACGC	
16251				ACAGCGCGCT TGTCGCGCGA	
16301				TGCAGATATG ACGTCTATAC	
16351				GAGGAAGAAT CTCCTTCTTA	
16401				GGCATGCGTC CCGTACGCAG	
16451				GCGCGGCGGT CGCGCCGCCA	
16501				GCGCCGTGCC	
16551				TTAAAAACAA AATTTTTGTT	•
16601				ACGCTCGCTT TGCGAGCGAA	
16651				GCGTCTCTGG CGCAGAGACC	
16701				AGATATCGGC TCTATAGCCG	
16751	TGAGCGGTGG ACTCGCCACC	CGCCTTCAGC GCGGAAGTCG	TGGGGCTCGC ACCCCGAGCG	TGTGGAGCGG ACACCTCGCC	CATTAAAAAT GTAATTTTTA
16801	TTCGGTTCCA AAGCCAAGGT	CCGTTAAGAA GGCAATTCTT	CTATGGCAGC GATACCGTCG	AAGGCCTGGA TTCCGGACCT	ACAGCAGCAC TGTCGTCGTG
16851	AGGCCAGATG TCCGGTCTAC	CTGAGGGATA GACTCCCTAT	AGTTGAAAGA TCAACTTTCT	GCAAAATTTC CGTTTTAAAG	CAACAAAAGG GTTGTTTTCC
16901	TGGTAGATGG ACCATCTACC	CCTGGCCTCT GGACCGGAGA	GGCATTAGCG CCGTAATCGC	GGGTGGTGGA CCCACCACCT	CCTGGCCAAC GGACCGGTTG
16951					GCCCTCCCGT CGGGAGGGCA

Figure 27R

17001	AGAGGAGCCT TCTCCTCGGA	ceccec cancecce	TGGAGACAGT ACCTCTGTCA	GTCTCCAGAG CAGAGGTCTC	GGGCGTY G
17051	AAAAGCGTCC	GCGCCCCGAC	AGGGAAGAAA	CTCTGGTGAC	GCAAATAGAC
	TTTTCGCAGG	CGCGGGGCTG	TCCCTTCTTT	GAGACCACTG	CGTTTATCTG
17101	GAGCCTCCCT	CGTACGAGGA	GGCACTAAAG	CAAGGCCTGC	CCACCACCCG
	CTCGGAGGGA	GCATGCTCCT	CCGTGATTTC	GTTCCGGACG	GGTGGTGGGC
17151	TCCCATCGCG AGGGTAGCGC	CCCATGGCTA GGGTACCGAT	CCGGAGTGCT GGCCTCACGA	GGGCCAGCAC	ACACCCGTAA TGTGGGCATT
17201	CGCTGGACCT	GCCTCCCCCC	GCCGACACCC	AGCAGAAACC	TGTGCTGCCA
	GCGACCTGGA	CGGAGGGGGG	CGCCTGTGGG	TCGTCTTTGG	ACACGACGGT
17251	GGCCCGACCG CCGGGCTGGC	CCGTTGTTGT GGCAACAACA	AACCCGTCCT TTGGGCAGGA	AGCCGCGCGT TCGGCGCGCA	CCCTGCGCCGC
17301	CGCCGCCAGC	GGTCCGCGAT	CGTTGCGGCC	CGTAGCCAGT	GGCAACTGGC
	GCGGCGGTCG	CCAGGCGCTA	GCAACGCCGG	GCATCGGTCA	CCGTTGACCG
17351	AAAGCACACT	GAACAGCATC	GTGGGTCTGG	GGGTGCAATC	CCTGAAGCGC
	TTTCGTGTGA	CTTGTCGTAG	CACCCAGACC	CCCACGTTAG	GGACTTCGCG
17401	CGACGATGCT	TCTGATAGCT	AACGTGTCGT	ATGTGTGTCA	TCTATCCGTC
	GCTGCTACGA	AGACTATCGA	TTGCACAGCA	TACACACAGT	ACATACGCAG
17451	CATGTCGCCG GTACAGCGGC	CCAGAGGAGC GGTCTCCTCG	TGCTGAGCCG ACGACTCGGC	GCGCGCGCCC	GCTTTCCAAG CGAAAGGTTC
17501	ATGGCTACCC	CTTCGATGAT	GCCGCAGTGG	TCTTACATGC	ACATCTCGGG
	TACCCATGGG	GAAGCTACTA	CGGCGTCACC	AGAATGTACG	TGTAGAGCCC
17551	CCAGGACGCC	TCGGAGTACC	TGAGCCCCGG	GCTGGTGCAG	TTTGCCCGCG
	GGTCCTGCGG	AGCCTCATGG	ACTCGGGGCC	CGACCACGTC	AAACGGGCGC
17601	CCACCGAGAC	GTACTTCAGC	CTGAATAACA	AGTTTAGAAA	CCCCACGGTG
	GGTGGCTCTG	CATGAAGTCG	GACTTATTGT	TCAAATCTTT	GGGGTGCCAC
17651	GCGCCTACGC	ACGACGTGAC	CACAGACCGG	TCCCAGCGTT	TGACGCTGCG
	CGCGGATGCG	TGCTGCACTG	GTGTCTGGCC	AGGGTCGCAA	ACTGCGACGC
17701	GTTCATCCCT	GTGGACCGTG	AGGATACTGC	GTACTCGTAC	AAGGCGCGGT
	CAAGTAGGGA	CACCTGGCAC	TCCTATGACG	CATGAGCATG	TTCCGCGCCA
17751	TCACCCTAGC	TGTGGGTGAT	AACCGTGTGC	TGGACATGGC	TTCCACGTAC
	AGTGGGATCG	ACACCCACTA	TTGGCACACG	ACCTGTACCG	AAGGTGCATG
17801	TTTGACATCC	GCGGCGTGCT	GGACAGGGGC	CCTACTTTTA	AGCCCTACTC
	AAACTGTAGG	CGCCGCACGA	CCTGTCCCCG	GGATGAAAAT	TCGGGATGAG
17851	TGGCACTGCC	TACAACGCCC	TGGCTCCCAA	GGGTGCCCCA	AATCCTTGCG
	ACCGTGACGG	ATGTTGCGGG	ACCGAGGGTT	CCCACGGGGT	TTAGGAACGC
17901	AATGGGATGA	AGCTGCTACT	GCTCTTGAAA	TAAACCTAGA	AGAAGAGGAC
	TTACCCTACT	TCGACGATGA	CGAGAACTTT	ATTTGGATCT	TCTTCTCCTG

Figure 275

17951	GATGACAACG CTACTGTTGC	A CGAAGT TTCTGCTTCA		
18001	CGTATTTGGG GCATAAACCC	CAGGCGCCTT GTCCGCGGAA		
18051		TGTCGAAGGT ACAGCTTCCA		
18101	CAACCTGAAC GTTGGACTTG	CTCAAATAGG GAGTTTATCC	-	
18151		GGGAGAGTCC CCCTCTCAGG		
18201	ACGGTTCATA TGCCAAGTAT	TGCAAAACCC ACGTTTTGGG		
18251		AAAATGGAAA TTTTACCTTT		
18301	• - •	GAGGCAGCCG CTCCGTCGGC		
18351		CAGTGAAGAT GTCACTTCTA		
18401		CCACTATTAA GGTGATAATT		
18451		CCCAACAGGC GGGTTGTCCG		
18501		GTATTACAAC CATAATGTTG		
18551		AGTIGAATGC TCAACTTACG		
18601		CAGCTTTTGC GTCGAAAACG		
18651		GAATCAGGCT CTTAGTCCGA		
18701	ATTGAAAATC TAACTTTTAG	ATGGAACTGA TACCTTGACT		
18751	GGGAGGTGTG CCCTCCACAC	ATTAATACAG TAATTATGTC		
18801	GTCAGGAAAA CAGTCCTTTT	TGGATGGGAA ACCTACCCTT		
18851	GAAATAAGAG CTTTATTCTC	TTGGAAATAA AACCTTTATT		

Figure 27T

18901	CCTGTGGAGA GGACACCTCT	A TCCTGT TTAAAGGACA	ACTCCAACAT TGAGGTTGTA	AGCGCTGTAT TCGCGACATA	AACGGGCTGT
18951	AGCTAAAGTA TCGATTTCAT	CAGTCCTTCC GTCAGGAAGG	AACGTAAAAA TTGCATTTTT	TTTCTGATAA AAAGACTATT	CCCAAACACC GGGTTTGTGG
19001				CCCGGGCTAG GGGCCCGATC	
19051				CTATATGGAC GATATACCTG	
19101	GTAAATTGGT	GGTGGCGTTA	CGACCGGACG	GCTACCGCTC CGATGGCGAG	TTACAACGAC
19151	CCGTTACCAG	CGATACACGG	GAAGGTGTAG	CAGGTGCCTC GTCCACGGAG	TCTTCAAGAA
19201	ACGGTAATTT	TTGGAGGAAG	AGGACGGCCC	CTCATACACC GAGTATGTGG	ATGCTCACCT
19251	TGAAGTCCTT	CCTACAATTG	TACCAAGACG	AGAGCTCCCT TCTCGAGGGA	TCCTTTACTG
19301	GATTCCCAAC	TGCCTCGGTC	GTAATTCAAA	GATAGCATTT CTATCGTAAA	CGGAAATGCG
19351	GTGGAAGAAG	GGGTACCGGG	TGTTGTGGCG	CTCCACGCTT GAGGTGCGAA	CTCCGGTACG
19401	AATCTTTGCT	GTGGTTGCTG	GTCAGGAAAT	ACGACTATCT TGCTGATAGA	CAGGCGGCGG
19451	TTGTACGAGA	TGGGATATGG	GCGGTTGCGA	ACCAACGTGC TGGTTGCACG	GGTATAGGTA
19501	GGGGAGGGCG	TTGACCCGCC	GAAAGGCGCC	CTGGGCCTTC GACCCGGAAG	TGCGCGGAAT
19551				GCTACGACCC CGATGCTGGG	
19601	TACTCTGGCT ATGAGACCGA	CTATACCCTA GATATGGGAT	CCTAGATGGA GGATCTACCT	ACCTTTTACC TGGAAAATGG	TCAACCACAC AGTTGGTGTG
19651	CTTTAAGAAG GAAATTCTTC	GTGGCCATTA CACCGGTAAT	CCTTTGACTC GGAAACTGAG	TTCTGTCAGC AAGACAGTCG	TGGCCTGGCA ACCGGACCGT
		CGAATGGGGG	TTGCTCAAAC	TTTAATTCGC	GAGTCAACTG
19751	GGGGAGGGTT CCCCTCCCAA	ACAACGTTGC TGTTGCAACG	CCAGTGTAAC GGTCACATTG	ATGACCAAAG TÄCTGGTTTC	ACTGGTTCCT TGACCAAGGA
19801	GGTACAAATG CCATGTTTAC	CTAGCTAACT GATCGATTGA	ATAACATTGG TATTGTAACC	CTACCAGGGC GATGGTCCCG	TTCTATATCC AAGATATAGG

Figure 274

19851	CAGACAGCTA GTCTCTCGAT			CTTCCAC
19901	ATGAGCCGTC TACTCGGCAG			ACCAACAGGT TGGTTGTCCA
19951				TACCTTGCCC ATGGAACGGG
20001				CTATCCGCTT GATAGGCGAA
20051				TTCTTTGCGA AAGAAACGCT
20101			 TAACTTTATG ATTGAAATAC	TCCATGGGCG AGGTACCCGC
20151			ACGCCAACTC TGCGGTTGAG	
20201			GACGAGCCCA CTGCTCGGGT	
20251			TGTGCACCAG ACACGTGGTC	
20301			CCTTCTCGGC GGAAGAGCCG	
20351			CAGCTGCCGC GTCGACGGCG	
20401			GATCTTGGTT CTAGAACCAA	
20451			AGGCTTTGTT TCCGAAACAA	
20501			GTCGCGAGAC CAGCGCTCTG	
20551			TCAAAAACAT AGTTTTTGTA	
20601	TGAGCCCTTT ACTCGGGAAA		CAAGCAGGTT GTTCGTCCAA	
20651	AGTACGAGTC TCATGCTCAG		TTGCTTCTTC AACGAAGAAG	
20701	TGTATAACGC ACATATTGCG		GTACAGGGGC CATGTCCCCG	
20751	CGCCTGTGGA GCGGACACCT	-	CCACGCCTTT GGTGCGGAAA	

Figure 27 V.

20801	CCCAAACTCC GGGTTTGAGG	C GATCAC GTACCTAGTG	AACCCCACCA TTGGGGTGGT	TGAACCTTAT ACTTGGAATA	TACCGG AT
20851				CAGCCCACCC GTCGGGTGGG	
20901	GGTCCTTGTC	GAGATGTCGA	AGGACCTCGC	CCACTCGCCC GGTGAGCGGG	ATGAAGGCGT
20951				CTTTTTGTCA GAAAAACAGT	
21001	TACATTTTTA	TTACATGATC	TCTGTGAAAG	AATAAAGGCA TTATTTCCGT	TTACGAAAAT
21051	AAACATGTGA	GAGCCCACTA	ATAAATGGGG	CACCCTTGCC GTGGGAACGG	CAGACGCGGC
21101	AAATTTTTAG	TTTCCCCAAG	ACGGCGCGTA	CGCTATGCGC GCGATACGCG	GTGACCGTCC
21151	CTGTGCAACG	CTATGACCAC	AAATCACGAG	CACTTAAACT GTGAATTTGA	GTCCGTGTTG
21201	GTAGGCGCCG	TCGAGCCACT	TCAAAAGTGA	CCACAGGCTG GGTGTCCGAC	GCGTGGTAGT
21251	GGTTGCGCAA	ATCGTCCAGC	CCGCGGCTAT	TCTTGAAGTC AGAACTTCAG	CGTCAACCCC
21301	GGAGGCGGGA	CGCGCGCGCT	CAACGCTATG	ACAGGGTTGC TGTCCCAACG	TCGTGACCTT
21351	GTGATAGTCG	CGGCCCACCA	CGTGCGACCG	CAGCACGCTC GTCGTGCGAG	AACAGCCTCT
21401	AGTCTAGGCG	CAGGTCCAGG	AGGCGCAACG	TCAGGGCGAA AGTCCCGCTT	GCCTCAGTTG
21451	AAACCATCGA	CGGAAGGGTT	TTTCCCGCGC	TGCCCAGGCT ACGGGTCCGA	AACTCAACGT
21501	GAGCGTGGCA	TCACCGTAGT	TTTCCACTGG	GTGCCCGGTC CACGGGCCAG	ACCCGCAATC
		GACGTATTTT	CGGAACTAGA	CGAATTTTCG	GTGGACTCGG
	AAACGĊGGAA	GTCTCTTCTT	GTACGGCGTT	CTGAACGGCC	AAAACTGATT TTTTGACTAA
21651	GGCCGGACAG CCGGCCTGTC	GCCGCGTCGT	GCACGCAGCA CGTGCGTCGT	CCTTGCGTCG GGAACGCAGC	GTGTTGGAGA CACAACCTCT
21701	TCTGCACCAC AGACGTGGTG	ATITCGGCCC TAAAGCCGGG	CACCGGTTCT	TCACGATCTT AGTGCTAGAA	GGCCTTGCTA CCGGAACGAT

7, gure 27 W

21751		TCGCGCGC AGTCGCGCGC			
21801	AATCACGTGC TTAGTGCACG	TCCTTATTTA AGGAATAAAT			
21851		CTCAGCGCAG GAGTCGCGTC			
21901		TGTAGGTCAC ACATCCAGTG			
21951		ATCATCGTCA TAGTAGCAGT		-	
22001		GTGCTCCTCG CACGAGGAGC			
22051	GCTTCCACTT CGAAGGTGAA	GGTCAGGCAG CCAGTCCGTC	TAGTTTGAAG ATCAAACTTC	TTCGCCTTTA AAGCGGAAAT	GATCGTTATC CTAGCAATAG
22101		TTGTCCATCA AACAGGTAGT			
22151		GATCGGCACA CTAGCCGTGT			
22201		TGGGCTCTTC ACCCGAGAAG			
22251		TCTTCATTCA AGAAGTAAGT			
22301		TAGCACCGGT ATCGTGGCCA			
22351		TTTCTTCCTC AAAGAAGGAG			
22401		TTGGGAGAAG AACCCTCTTC			
22451		CGCCGAGGTC GCGGCTCCAG			
22501	AGCGCGTCTT TCGCGCAGAA	GTGATGAGTC CACTACTCAG			
		AAACCCCCGC	GGGCCCCTCC	GCCGCCGCTG	CCCCTGCCCC
		GAGGTACCAA	CCCCTGCAG	CGCGGCGTGG	CGCAGGCGCG
22651	TCGGGGGTGG AGCCCCCACC	TTTCGCGCTG AAAGCGCGAC			

Figure 27X

22701	CTATAGGCAG GATATCCGTC	A GATCA TTTTTCTAGT	TGGAGTCAGT ACCTCAGTCA	CGAGAAGAAG GCTCTTCTTC	GACAGCT A CTGTCGGATT
22751				CCACCGATGC GGTGGCTACG	
22801				CTTGAGGAGG GAACTCCTCC	
22851				AGACGACGAG TCTGCTGCTC	
22901				ACAACGCAGA TGTTGCGTCT	
22951				GGCGACTACC CCGCTGATGG	
23001				CCAGTGCGCC GGTCACGCGG	
23051				TCGCCATAGC AGCGGTATCG	
23101	CTTGCCTACG GAACGGATGC			CGCGTACCCC GCGCATGGGG	
23151				CCTCAACTTC GGAGTTGAAG	
23201				ACATCTTTTT TGTAGAAAAA	
23251				AGCCGAGCGG TCGGCTCGCC	
23301				TATCGCCTCG ATAGCGGAGC	
23351				ACGAGAAGCG TGCTCTTCGC	
23401				AGTCACTCTG TCAGTGAGAC	
23451	GGAACTCGAG CCTTGAGCTC	GGTGACAACG CCACTGTTGC	CGCGCCTAGC GCGCGGATCG	CGTACTAAAA GCATGATTTT	CGCAGCATCG GCGTCGTAGC
23501	AGGTCACCCA TCCAGTGGGT	CTTTGCCTAC GAAACGGATG	CCGGCACTTA GGCCGTGAAT	ACCTACCCC TGGATGGGGG	CAAGGTCATG GTTCCAGTAC
23551	AGCACAGTCA TCGTGTCAGT	TGAGTGAGCT ACTCACTCGA	GATCGTGCGC CTAGCACGCG	CGTGCGCAGC GCACGCGTCG	CCCTGGAGAG GGGACCTCTC
23601	GGATGCAAAT CCTACGTTTA	TTGCAAGAAC AACGTTCTTG	AAACAGAGGA TTTGTCTCCT	GGGCCTACCC CCCGGATGGG	GCAGTTGGCG CGTCAACCGC

Figure 27 Y

23651					CGACTT G
23701					TGGAGCTTGA
23701					ACCTCGAACT
23751					AAGCTAGAGG TTCGATCTCC
	CACGIACGIC	GCCAAGAAAC	. GACIGGGCCI	CINCGICGCG	TICGATCICC
23801			-		GCCTGCAAG
	TTTGTAACGT	GATGTGGAAA	GCTGTCCCGA	TGCATGCGGT	CCGGACGTTC
23851					GAATTTTGCA
	TAGAGGTTGC	ACCTCGAGAC	GTTGGACCAG	AGGATGGAAC	CTTAAAACGT
23901	CGAAAACCGC	CTTGGGCAAA	ACGTGCTTCA	TTCCACGCTC	AAGGGCGAGG
	GCTTTTGGCG	GAACCCGTTT	TGCACGAAGT	AAGGTGCGAG	TTCCCGCTCC
23951	CGCGCCGCGA	CTACGTCCGC	GACTGCGTTT	ልርጥጥልጥጥጥ ተ	ATGCTACACC
					TACGATGTGG
24001	macas es coc	COMPOSSO	mmcca x co x c	moommoooo	AGTGCAACCT
2400T					TCACGTTGGA
24051			TAAAGCAAAA		
	GTTCCTCGAC	GTCTTTGACG	ATTTCGTTTT	GAACTTCCTG	GATACCTGCC
24101			GCCGCGCACC		
	GGAAGTTGCT	CGCGAGGCAC	CGGCGCGTGG	ACCGCCTGTA	GTAAAAGGGG
24151	GAACGCCTGC	TTAAAACCCT	GCAACAGGGT	CTGCCAGACT	TCACCAGTCA
	CTTGCGGACG	AATTTTGGGA	CGTTGTCCCA	GACGGTCTGA	AGTGGTCAGT
24201			GGAACTTTAT		
	TTCGTACAAC	GTCTTGAAAT	CCTTGAAATA	GGATCTCGCG	AGTCCTTAGA
24251	TGCCCGCCAC	CTGCTGTGCA	CTTCCTAGCG	ACTTTGTGCC	CATTAAGTAC
	ACGGGCGGTG	GACGACACGT	GAAGGATCGC	TGAAACACGG	GTAATTCATG
24301	CGCGAATGCC	CTCCGCCGCT	TTGGGGCCAC	TGCTACCTTC	TGCAGCTAGC
	GCGCTTACGG	GAGGCGGCGA	AACCCCGGTG	ACGATGGAAG	ACGTCGATCG
24351	CAACTACCTT	CCCTACCACT	CTGACATAAT	CCAACACCTC	AGCGGTGACG
24334			GACTGTATTA		
24401	GTCTACTGGA	GTGTCACTGT	CGCTGCAACC	TATGCACCCC	GCACCGCTCC
	CAGATGACCT	CACAGTGACA	GCGACGTIGG	atacgtgggg	CGTGGCGAGG
24451	CTGGTTTGCA	አ ባማርርር ልርርጥ	GCTTAACGAA	<u>እርጥር ል አጥጥ አ</u>	ጥ ሶርር ርጥል
	GACCAAACGT				
24503	mas cameas s	0000000000	CDCDCCC	00000000 00	000000
24501	TGAGCTGCAG ACTCGACGTC				
24551	AACTCACTCC				
	TTGACTGAGG	CCCCGACACC	TGCAGCCGAA	TUGAAGCGTT	TAAACATGGA

Figure 27Z

24601		ACCCCACGA TGCGGGTGCT			
24651		GAGCTTACCG CTCGAATGGC			
24701		AGCCATCAAC TCGGTAGTTG			
24751		TTTACTTGGA AAATGAACCT			
24801		CCGCAGCCCT GGCGTCGGGA			
24851		CCAAAAAGAA GGTTTTTCTT			
24901		TGGGACAGTC ACCCTGTCAG			
24951		GGAAGACTGG CCTTCTGACC			
25001		CAGACGAAAC GTCTGCTTTG			
25051		AAATCGGCAA TTTAGCCGTT			
25101		GCCGGCACTG CGGCCGTGAC			
25151		CCAGGGCCGG			
25201		CAGCGCCAAG GTCGCGGTTC			
25251		TTGCTTGCAA AACGAACGTT			
25301		TCTACCATCA AGATGGTAGT			
		CATCTCTACA GTAGAGATGT			
25401		CCACACAGAA GGTGTGTCTT			
25451		AAATCCACAG TTTAGGTGTC			
25501	GTCTGGCGCC CAGACCGCGG	CAACGAACCC GTTGCTTGGG	GTATCGACCC CATAGCTGGG	GCGAGCTTAG CGCTCGAATC	AAACAGGATT TTTGTCCTAA

Figure 27. AA

25551	TTTCCCACTC AAAGGGTGAG	TGCTAT ACATACGATA		
25601		AAAAACAGGT TTTTTGTCCA		
25651		CGAAGATCAG GCTTCTAGTC		
25701		AATACTGCGC TTATGACGCG		
25751	TTCTCAAATT AAGAGTTTAA	TAAGCGCGAA ATTCGCGCTT		
25801		TGTTGTCAGC ACAAÇAGTCG		
25851	TACATGTGGA ATGTACACCT	GTTACCAGCC CAATGGTCGG		
25901		ACCCGAATAA TGGGCTTATT	 	
25951		CGGAATACGC GCCTTATGCG		
26001		CCACCACACC GGTGGTGTGG		
26051		GTGTACCAGG CACATGGTCC		
26101		CCAGGCCGAA GGTCCGGCTT		
26151		TTCGTCACAG AAGCAGTGTC		
26201		AGAGGGCGAG TCTCCCGCTC		
26251		TCTCCGTCCG AGAGGCAGGC		
26301	CGCTCTTCAT GCGAGAAGTA	TCACGCCTCG AGTGCGGAGC		
26351	CTCTGAGCCG GAGACTCGGC			
26401	TTGTGCCATC AACACGGTAG			
26451	CCGGATCAAT GGCCTAGTTA			

Figure 27 AB

26501	CTACGACTGA GATGCTGACT	A TAAGTG TACAATTCAC	GAGAGGCAGA CTCTCCGTCT	GCAACTGCGC CGTTGACGCG	CTGAAA CC GACTTTGTGG
26551	TGGTCCACTG ACCAGGTGAC	TCGCCGCCAC AGCGGCGGTG	AAGTGCTTTG TTCACGAAAC	CCCGCGACTC GGGCGCTGAG	CGGTGAGTTT GCCACTCAAA
26501	ACGATGAAAC	TTAACGGGCT	GGATCATATC CCTAGTATAG	CTCCCGGGCC	GCGTGCCGCA
26651	GGCCGAATGG	CGGGTCCCTC	AGCTTGCCCG TCGAACGGGC	ATCGGACTAA	GCCCTCAAAT
26701	GGGTCGCGGG	GGACGATCAA	GAGCGGGACA CTCGCCCTGT	CCCCTGGGAC	ACAAGAGTGA
26751	CACTAAACGT	TGACAGGATT	CCCTGGATTA GGGACCTAAT	GTAGTTCTAG	AAACAACGGT
26801	AGAGACACGA	CTCATATTAT	AATACAGAAA TTATGTCTTT	AATTTTATAT	GACCCCGAGG
26851	ATAGCGGTAG	GACATTTGCG	CACCGTCTTC GTGGCAGAAG	TGGGCGGGTT	CETTTGGTTC
26901	CGCTTGGAAT	GGACCATGAA	TTAACATCTC AATTGTAGAG	AGGGAGACAC	TAAATGTTGT
26951	CAAAGTTGGG	TCTGCCTCAC	AGTCTACGAG TCAGATGCTC	TCTTGGAGAG	GCTCGAGTCG
27001	ATGAGGTAGT	CTTTTTTGTG	CACCCTCCTT	TGGACGGCCC	TTGCATGCTC
27051	ACGCAGTGGC	CGGCGACGTG	CACACCTACC GTGTGGATGG	CGGACTGGCA	TTTGGTCTGA
27101	AAAAGGCCTG	TCTGGAGTTA	AACTCTGTTT TTGAGACAAA	TGGTCTTGTC	CTCCACTCGA
27151	ATCTTTTGGG	AATCCCATAA	AGGCCAAAGG TCCGGTTTCC	GCGTCGATGA	CACCCCAAAT
27201	ACTTGTTAAG	TTCGTTGAGA	ACGGGCTATT TGCCCGATAA	GATTAAGTCC	AAAGAGATCT
		CCCAATAAGA	GACAGAACAC	TAAGAGAAAT	AAGAATATGA
		ACGGATTCCG	AGCGGCGGAC	GACACACGTG	TAAACGTAAA
		AAAATTTGCG	ACCCCAGCGG	TGGGTTCTAC	TAATCCATGT
27401	TAATCCTAGG ATTAGGATCC	TTTACTCACC AAATGAGTGG	CTTGCGTCAG GAACGCAGTC	CCCACGGTAC	CACCCAAAAG

Figure 27AC

27451			CTGAAG. A GACTTCGATT
27501	_		AAGCTGCTTA TTCGACGAAT
27551		 GGCAAGTATG CCGTTCATAC	
27601		 TAATGTTACA ATTACAATGT	 GTAAAAGTCA CATTTTCAGT
27651		 TTCCATTTTA AAGGTAAAAT	 GACATTACCA CTGTAATGGT
27701		AAGTTGTGGC TTCAACACCG	TTGTGTGGAA AACACACCTT
27751		CACTGCTATG GTGACGATAC	
27801		TTAAATACAA AATTTATGTT	
27851		TTTACTAAGT AAATGATTCA	
27901		 TGCAAAACAA ACGTTTTGTT	
27951		 CCCCCGTCA	
28001		CTGGGATATG CACCCTATAC	
28051		 AGCATCTGAC TCGTAGACTG	
28101		 TACAGCGACC ATGTCGCTGG	
28151		GCTACCGGAC CGATGGCCTG	
28201	CCCCAAGTTT GGGGTTCAAA	CAATAACTGG GTTATTGACC	
28251	GTTCTCCATA CAAGAGGTAT	 TTGTATGCCT AACATACGGA	
28301	GCTGCCTAAA CGACGGATTT		TCCCATCATT AGGGTAGTAA
28351	GTGCTACACC CACGATGTGG		

Figure 27AD

28401	CATGTTCTTT GTACAAGAAA	TTACAG AGAGAATGTC	TATGATTAAA ATACTAATTT	TGAGACATGÁ ACTCTGTACT	TTCCTC T AAGGAGCTCA
28451	TTTTATATTA AAAATATAAA	CTGACCCTTG GACTGGGAAC	TTGCGCTTTT AACGCGAAAA	TTGTGCGTGC AACACGCACG	TCCACATTGG AGGTGTAACC
28501	CTGCGGTTTC GACGCCAAAG	TCACATCGAA AGTGTAGCTT	GTAGACTGCA CATCTGACGT	TTCCAGCCTT AAGGTCGGAA	CACAGTCTAT GTGTCAGATA
28551	TTGCTTTACG AACGAAATGC	GATTTGTCAC CTAAACAGTG	CCTCACGCTC GGAGTGCGAG	ATCTGCAGCC TAGACGTCGG	TCATCACTGT AGTAGTGACA
28601	CCAGTAGCGG	AAATAGGTCA	GCATTGACTG CGTAACTGAC	CCAGACACAC	GCGAAACGTA
28651	TAGAGTCTGT	GGTAGGGGTC	TACAGGGACA ATGTCCCTGT	CCTGATATCG	ACTCGAAGAA
28701	TCTTAAGAAA	TTAATACTTT	TTTACTGTGA AAATGACACT	GAAAAGACGA	CTAATAAACG
28751	TGGGATAGAC	GCAAAACAAG	CCCGACCTCC GGGCTGGAGG	TTCGGAGTTT	CTGTATATAG
28801	TACGTCTAAG	TGAGCATATA	GGAATATTCC CCTTATAAGG	TTCAACGATG	TTACTTTTTT
28851	CGCTAGAAAG	GCTTCGGACC	TTATATGCAA AATATACGTT	AGTAGAGACA	ATACCACAAG
28901	ACGTCATGGT	AGAATCGGGA	AGCTATATAT TCGATATATA	GGGATGGAAC	TGTAACCGAC
28951	CTTGCGTTAT	CTACGGTACT	ACCACCCAAC TGGTGGGTTG	AAAGGGGCGC	GGGCGATACG
29001	AAGGTGACGT	TGTTCAACAA	CGGCCGCCGA	AACAGGGTCG	CAATCAGCCT GTTAGTCGGA
29051	GCGGGTGGAA	GAGGGTGGGG	GTGACTTTAG	TCGATGAAAT	ATCTAACAGG TAGATTGTCC
29101	TCCTCTACTG	ACTGTGGGAT	CTAGATCITT	ACCTGCCTTA	TATTACAGAG ATAATGTCTC
	GTCGCGGACG	ATCTTTCTGC	GTCCCGTCGC	CGGCTCGTTG	AGCGCATGAA TCGCGTACTT
	AGTTCTCGAG	GTTCTGTACC	AATTGAACGT	GGTCACGTTT	AGGGGTATCT TCCCCATAGA
	AAACAGAGCA	TTTCGTCCGG	TTTCAGTGGA	TGCTGTCATT	TACCACCGGA ATGGTGGCCT
29301	CACCGCCTTA GTGGCGGAAT	GCTACAAGTT CGATGTTCAA	GCCAACCAAG	CGTCAGAAAT GCAGTCTTTA	TGGTGGTCAT

Figure 27 AE

29351				GAAACC
29401			-	 CACCCTTATT GTGGGAATAA
29451	AAGACCCTGT TTCTGGGACA	GCGGTCTCAA CGCCAGAGTT		
29501		CATCACTTAC GTAGTGAATG		TCTGTCCAGT AGACAGGTCA
29551		GCACCTCCTT CGTGGAGGAA		ATTGCAGCTT TAACGTCGAA
29601		GCAAACTTTC CGTTTGAAAG		
29651		TCCATCCGCA AGGTAGGCGT		
29701	•••	CGTCTGAAGA GCAGACTTCT		
29751		CCTCCAACTG GGAGGTTGAC	-	
29801		TCAAGAGAGT AGTTCTCTCA		
29851		TTACCTCCAA AATGGAGGTT		
29901		GACGAGGCCG CTGCTCCGGC		_
29951		TCTCAAAAAA AGAGTTTTTT		
30001		CAGTTACCTC GTCAATGGAG		
30051		GCGGGCAACA CGCCCGTTGT		
30101	CCGTGCACGA GGCACGTGCT	CTCCAAACTT GAGGTTTGAA		
30151	TCAGAAGGAA AGTCTTCCTT			
	TAGCAGTACC ATCGTCATGG			
30251	GTAGCTTGGG CATCGAACCC			

Figure 27 AF

30301	CTAGGACTAA GATCCTGATT	A CGGGGC	TCCTTTGCAT AGĠAAACGTA	GTAACAGAČG CATTGTCTGC	TACCTAATCC TGGATTTCTG
30351				TATTAATAAT ATAATTATTA	
30401				ATTCACAAGG TAAGTGTTCC	
30451				TCTCAAAACA AGAGTTTTGT	
30501	ACTTGATGTT TGAACTACAA			AAACCAACTA TTTGGTTGAT	
30551				CCCACAACTT GGGTGTTGAA	
30601				TCAAACAATT AGTTTGTTAA	
30651				GATGTTTGAC CTACAAACTG	
30701				TTGGTTCACC AACCAAGTGG	
30751				CATGGCCTAG GTACCGGATC	
30801				TGGCCTTAGT ACCGGAATCA	
30851				ATGATAAGCT TACTATTCGA	
30901				CTAAATGCAG GATTTACGTC	
30951				CAGTCAAATA GTCAGTTTAT	
31001				CTCCAATATC GAGGTTATAG	
31051	CAAAGTGCTC GTTTCACGAG	ATCTTATTAT TAGAATAATA	AAGATTTGAC TTCTAAACTG	GAAAATGGAG CTTTTACCTC	TGCTACTAAA ACGATGATTT
31101	CAATTCCTTC GTTAAGGAAG	CTGGACCCAG GACCTGGGTC	AATATTGGAA TTATAACCTT	CTTTAGAAAT GAAATCTTTA	GGAGATCTTA CCTCTAGAAT
31151	CTGAAGGCAC GACTTCCGTG	AGCCTATACA TCGGATATGT	AACGCTGTTG TTGCGACAAC	GATTTÀTGCC CTAAATACGG	TAACCTATCA ATTGGATAGT
31201	GCTTATCCAA CGAATAGGTT	AATCTCACGG TTAGAGTGCC	TAAAACTGCC ATTTTGACGG	AAAAGTAACA TTTTCATTGT	TTGTCAGTCA AACAGTCAGT

Figure 27 AG

31251			AAACTAAACC TTTGATTTGG		
31301	TAAACGGTAC ATTTGCCATG		GGAGACACAA CCTCTGTGTT		
31351	TCATTTTCAT AGTAAAAGTA		TGGCCACAAC ACCGGTGTTG		
31401			CATACATTGC GTATGTAACG		
31451			TATTTTTCAA ATAAAAAGTT		
31501	TTTTTCATTC AAAAAGTAAG		CCCCACCACC GGGGTGGTGG		
31551			AGAACCCTAG TCTTGGGATC		
31601			AGTCCTTTCT TCAGGAAAGA		
31651			ACATATTCTT TGTATAAGAA		
31701			TCATCAGTGA AGTAGTCACT		
31751			GCTGTCCAGC CGACAGGTCG		
31801			CGGGCGGCGA GCCCGCCGCT		
31851			TGCATCAGGA ACGTAGTCCT		
31901			GGCGGCGC		
31951			CGATGATTCG GCTACTAAGC		
32001	GCCTTGTCCT CGGAACAGGA	CCGGGCACAG GGCCCGTGTC	CAGCGCACCC GTCGCGTGGG	TGATCTCACT ACTAGAGTGA	TAAATCAGCA ATTTAGTCGT
32051	CAGTAACTGC GTCATTGACG				CACAGTGCAA GTGTCACGTT
32101	GGCGCTGTAT CCGCGACATA	CCAAAGCTCA GGTTTCGAGT	TGGCGGGGAC ACCGCCCCTG	CACAGAACCC GTGTCTTGGG	ACGTGGCCAT TGCACCGGTA
32151	CATACCACAA GTATGGTGTT	GCGCAGGTAG CGCGTCCATC	ATTAAGTGGC TAATTCACCG	GACCCCTCAT CTGGGGAGTA	AAACACGCTG TTTGTGCGAC

Figure 27 AH

32201		CTCTTT AATGGAGAAA			
32251		CTCTGATTAA GAGACTAATT			
32301		AACCTGCCCG TTGGACGGGC			
32351		AGTGGAGAGC TCACCTCTCG			
32401		TCAATGTTGG AGTTACAACC			
32451	TCAGGATTAC AGTCCTAATG	AAGCTCCTCC TTCGAGGAGG	CGCGTTAGAA GCGCAATCTT	CCATATCCCA GGTATAGGGT	GGGAACAACC CCCTTGTTGG
32501		TCAGCGTAAA AGTCGCATTT			
32551		TGCATTGTCA ACGTAACAGT			
32601		GGTAGCGCGG CCATCGCGCC			
32651		GAGTGCGCCG CTCACGCGGC			
32701		GGAACGCCGG CCTTGCGGCC			
32751		GACAAACAGA CTGTTTGTCT			
32801		TAGTTGTAGT ATCAACATCA			
32851		GGGTTCTATG CCCAAGATAC			
32901		CCGCAGAATA GGCGTCTTAT			
32951	CTGCGAGTCA GACGCTCAGT	CACACGGGAG GTGTGCCCTC			
33001	TTTTTTTATT AAAAAAATAA	CCAAAAGATT GGTTTTCTAA	ATCCAAAACC TAGGTTTTGG	TCAAAATGAA AGTTTTACTT	GATCTATTAA CTAGATAATT
33051	GTGAACGCGC CACTTGCGCG	TCCCCTCCGG	TGGCGTGGTC ACCGCACCAG	AAACTCTACA TTTGAGATGT	GCCAAAGAAC CGGTTTCTTG
33101	AGATAATGGC TCTATTACCG	ATTTGTAAGA TAAACATTCT			

Figure 27 AI

33151	GCCCTCACGT CGGGAGTGCA	GTGGAC GTCACCTG	GTAAAGGCTA CATTTCCGAT	AACCCTTCAG TTGGGAAGTC	TGTGAAVITC CCACTITATAG
33201		ATTCCAGCAC TAAGGTCGTG			
33251		CAATATATCT GTTATATAGA			
33301		TCTGCTCCAG AGACGAGGTC			
33351		GCAAAAATTC CGTTTTTAAG			
33401		TTAACAAAA AATTGTTTTT			
33451		ATAATCGTGC TATTAGCACG			
33501		CCATGACAAA GGTACTGTTT			
33551		CTAACCAGCG GATTGGTCGC			
33601		ATGCAAGGTG TACGTTCCAC			
33651		GCACATCGTA CGTGTAGCAT			
33701		ACCACAGAAA TGGTGTCTTT			
33751		CATAAACACA GTATTTGTGT			
3380İ		TCTTACAACA AGAATGTTGT			
33851	ACTACGGCCA TGATGCCGGT	TGCCGGCGTG ACGGCCGCAC	ACCGTAAAAA TGGCATTTTT	AACTGGTCAC TTGACCAGTG	CGTGATTAAA GCACTAATTT
33901	AAGCACCACC TTCGTGGTGG	GACAGCTCCT CTGTCGAGGA	CGGTCATGTC GCCAGTACAG	CGGAGTCATA GCCTCAGTAT	ATGTAAGACT TACATTCTGA
33951	CGGTAAACAC GCCATTTGTG	ATCAGGTTGA TAGTCCAACT	TTCACATCGG AAGTGTAGCC	TCAGTGCTAA AGTCACGATT	AAAGCGACCG TTTCGCTGGC
34001	AAATAGCCCG TTTATCGGGC	GGGGAATACA CCCCTTATGT	TACCCGCAGG ATGGGCGTCC	CGTAGAGACA GCATCTCTGT	ACATTACAGC TGTAATGTCG
34051	CCCCATAGGA GGGGTATCCT	GGTATAACAA CCATATTGTT	AATTAATAGG TTAATTATCC	AGAGAAAAAC TCTCTTTTTG	ACATAAACAC TGTATTTGTG

Figure 27AJ

34101	CTGAAAAAACC	CONTROCCTA	GGCAAAATAG	CACCCTCCCG	A CONCENTRAL
	GACTTTTTGG	G. JACGGAT	CCGTTTTATC	GTGGGAGGGC	GAGGTC
34151	ACATACAGCG	CTTCCACAGC	GGCAGCCATA	ACAGTCAGCC	TTACCAGTAA
	TGTATGTCGC	GAAGGTGTCG	CCGTCGGTAT	TGTCAGTCGG	AATGGTCATT
34201	AAAAGAAAAC	СТАТТААААА	AACACCACTC	GACACGGCAC	CAGCTCAATC
	TTTTCTTTTG	GATAATTTTT	TTGTGGTGAG	CTGTGCCGTG	GTCGAGTTAG
34251	えたかく みに みたがた	TABABABCCC	CCAACTGCAG	AGCGAGTATA	TATAGGACTA
39231	TCAGTGTCAC	ATTTTTTCCC	GGTTCACGTC	TCGCTCATAT	ATATCCTGAT
34301	AAAAATGACG	ጥአክሮርርጥጥአአ	እርጥሮሮአሮኒኒኒ	AAACACCCAG	AAAACCGCAC
24201				TTTGTGGGTC	
34351	GCGAACCTAC	GCCCAGAAAC	GAAAGCCAAA	AAACCCACAA	CTTCCTCAAA
	CGCTTGGATG	CGGGTCTTTG	CTTTCGGTTT	TTTGGGTGTT	GAAGGAGTTT
34401				CTTCCCATTT	
	AGCAGTGAAG	GCAAAAGGGT	GCAATGCAGT	GAAGGGTAAA	ATICTTTTGA
34451	ልሮልልሞሞርርርል	ACACATACAA	GTTACTCCGC	CCTAAAACCT	ACGTCACCCG
34431	TGTTAAGGGT	TGTGTATGTT	CAATGAGGCG	GGATTTTGGA	TGCAGTGGGC
	•				
34501	CCCCGTTCCC	ACGCCCCGCG	CCACGTCACA	AACTCCACCC TTGAGGTGGG	CCTCATTATC
	GGGGCAAGGG	TGCGGGGCGC	GGTGCAGTGT	TIGAGGIGGG	GGAGIANIAG
					PacI
34551	ATATTGGCTT	CAATCCAAAA	TAAGGTATAT	TATTGATGAT	GTTAATTAAG
34551	ATATTGGCTT TATAACCGAA	CAATCCAAAA GTTAGGTTTT	TAAGGTATAT ATTCCATATA	TATTGATGAT ATAACTACTA	GTTAATTAAG CAATTAATTC
	TATAACCGAA	GTTAGGTTTT	ATTCCATATA	ATAACTACTA	CAATTAATTC
34551 34601	TATAACCGAA	GTTAGGTTTT TGCGACGCGA	ATTCCATATA GGCTGGATGG	TATTGATGAT ATAACTACTA CCTTCCCCAT GGAAGGGGTA	CAATTAATTC
	TATAACCGAA AATTCGGATC TTAAGCCTAG	GTTAGGTTTT TGCGACGCGA ACGCTGCGCT	ATTCCATATA GGCTGGATGG CCGACCTACC	ATAACTACTA CCTTCCCCAT GGAAGGGGTA	CAATTAATTC TATGATTCTT ATACTAAGAA
	TATAACCGAA AATTCGGATC TTAAGCCTAG CTCGCTTCCG	GTTAGGTTTT TGCGACGCGA ACGCTGCGCT GCGGCATCGG	ATTCCATATA GGCTGGATGG CCGACCTACC GATGCCCGCG	ATAACTACTA CCTTCCCCAT GGAAGGGGTA TTGCAGGCCA	CAATTAATTC TATGATTCTT ATACTAAGAA TGCTGTCCAG
34601	TATAACCGAA AATTCGGATC TTAAGCCTAG CTCGCTTCCG	GTTAGGTTTT TGCGACGCGA ACGCTGCGCT GCGGCATCGG	ATTCCATATA GGCTGGATGG CCGACCTACC GATGCCCGCG	ATAACTACTA CCTTCCCCAT GGAAGGGGTA	CAATTAATTC TATGATTCTT ATACTAAGAA TGCTGTCCAG
34601	TATAACCGAA AATTCGGATC TTAAGCCTAG CTCGCTTCCG GAGCGAAGGC GCAGGTAGAT	GTTAGGTTTT TGCGACGCGA ACGCTGCGCT GCGGCATCGG CGCCGTAGCC GACGACCATC	ATTCCATATA GGCTGGATGG CCGACCTACC GATGCCCGCG CTACGGGCGC AGGGACAGCT	ATAACTACTA CCTTCCCCAT GGAAGGGGTA TTGCAGGCCA AACGTCCGGT TCAAGGCCAG	CAATTAATTC TATGATTCTT ATACTAAGAA TGCTGTCCAG ACGACAGGTC CAAAAGGCCA
34601 34651	TATAACCGAA AATTCGGATC TTAAGCCTAG CTCGCTTCCG GAGCGAAGGC GCAGGTAGAT	GTTAGGTTTT TGCGACGCGA ACGCTGCGCT GCGGCATCGG CGCCGTAGCC GACGACCATC	ATTCCATATA GGCTGGATGG CCGACCTACC GATGCCCGCG CTACGGGCGC AGGGACAGCT	ATAACTACTA CCTTCCCCAT GGAAGGGGTA TTGCAGGCCA AACGTCCGGT	CAATTAATTC TATGATTCTT ATACTAAGAA TGCTGTCCAG ACGACAGGTC CAAAAGGCCA
34601 34651	TATAACCGAA AATTCGGATC TTAAGCCTAG CTCGCTTCCG GAGCGAAGGC GCAGGTAGAT CGTCCATCTA	GTTAGGTTTT TGCGACGCGA ACGCTGCGCT GCGGCATCGG CGCCGTAGCC GACGACCATC CTGCTGGTAG	ATTCCATATA GGCTGGATGG CCGACCTACC GATGCCCGCG CTACGGGCGC AGGGACAGCT TCCCTGTCGA	ATAACTACTA CCTTCCCCAT GGAAGGGGTA TTGCAGGCCA AACGTCCGGT TCAAGGCCAG AGTTCCGGTC	CAATTAATTC TATGATTCTT ATACTAAGAA TGCTGTCCAG ACGACAGGTC CAAAAGGCCA GTTTTCCGGT
34601 34651 34701	TATAACCGAA AATTCGGATC TTAAGCCTAG CTCGCTTCCG GAGCGAAGGC GCAGGTAGAT CGTCCATCTA GGAACCGTAA	GTTAGGTTTT TGCGACGCGA ACGCTGCGCT GCGGCATCGG CGCCGTAGCC GACGACCATC CTGCTGGTAG	ATTCCATATA GGCTGGATGG CCGACCTACC GATGCCCGCG CTACGGGCGC AGGGACAGCT TCCCTGTCGA TTGCTGGCGT	ATAACTACTA CCTTCCCCAT GGAAGGGGTA TTGCAGGCCA AACGTCCGGT TCAAGGCCAG	TATGATTCTT ATACTAAGAA TGCTGTCCAG ACGACAGGTC CAAAAGGCCA GTTTTCCGGT GCTCCGCCCC
34601 34651 34701 34751	TATAACCGAA AATTCGGATC TTAAGCCTAG CTCGCTTCCG GAGCGAAGGC GCAGGTAGAT CGTCCATCTA GGAACCGTAA CCTTGGCATT	GTTAGGTTTT TGCGACGCGA ACGCTGCGCT GCGGCATCGG CGCCGTAGCC GACGACCATC CTGCTGGTAG AAAGGCCGCG TTTCCGGCGC	ATTCCATATA GGCTGGATGG CCGACCTACC GATGCCCGCG CTACGGGCGC AGGGACAGCT TCCCTGTCGA TTGCTGGCGT AACGACCGCA	ATAACTACTA CCTTCCCCAT GGAAGGGGTA TTGCAGGCCA AACGTCCGGT TCAAGGCCAG AGTTCCGGTC TTTTCCATAG AAAAGGTATC	CAATTAATTC TATGATTCTT ATACTAAGAA TGCTGTCCAG ACGACAGGTC CAAAAGGCCA GTTTTCCGGT GCTCCGCCCC CGAGGCGGGG
34601 34651 34701 34751	TATAACCGAA AATTCGGATC TTAAGCCTAG CTCGCTTCCG GAGCGAAGGC GCAGGTAGAT CGTCCATCTA CGTACCGTAA CCTTGGCATT	GTTAGGTTTT TGCGACGCGA ACGCTGCGCT GCGGCATCGG CGCCGTAGCC GACGACCATC CTGCTGGTAG AAAGGCCGCG TTTCCGGCGC	ATTCCATATA GGCTGGATGG CCGACCTACC GATGCCCGCG CTACGGGCGC AGGGACAGCT TCCCTGTCGA TTGCTGGCGT AACGACCGCA TCGACGCTCA	ATAACTACTA CCTTCCCCAT GGAAGGGGTA TTGCAGGCCA AACGTCCGGT TCAAGGCCAG AGTTCCGGTC TTTTCCATAG AAAAGGTATC AGTCAGAGGT	CAATTAATTC TATGATTCTT ATACTAAGAA TGCTGTCCAG ACGACAGGTC CAAAAGGCCA GTTTTCCGGT GCTCCGCCCC CGAGGCGGGG
34601 34651 34701 34751 34801	TATAACCGAA AATTCGGATC TTAAGCCTAG CTCGCTTCCG GAGCGAAGGC GCAGGTAGAT CGTCCATCTA CGTCCATCTA CCTTGGCATT CCTGACGAGC GGACTGCTCG	GTTAGGTTTT TGCGACGCGA ACGCTGCGCT GCGGCATCGG CGCCGTAGCC GACGACCATC CTGCTGGTAG AAAGGCCGCG TTTCCGGCGC ATCACAAAAA TAGTGTTTTT	ATTCCATATA GGCTGGATGG CCGACCTACC GATGCCCGCG CTACGGGCGC AGGGACAGCT TCCCTGTCGA TTGCTGGCGT AACGACCGCA TCGACGCTCA AGCTGCGAGT	ATAACTACTA CCTTCCCCAT GGAAGGGGTA TTGCAGGCCA AACGTCCGGT TCAAGGCCAG AGTTCCGGTC TTTTCCATAG AAAAGGTATC AGTCAGAGGT TCAGTCTCCA	CAATTAATTC TATGATTCTT ATACTAAGAA TGCTGTCCAG ACGACAGGTC CAAAAGGCCA GTTTTCCGGT GCTCCGCCCC CGAGGCGGGG GGCGAAACCC CCGCTTTGGG
34601 34651 34701 34751 34801	TATAACCGAA AATTCGGATC TTAAGCCTAG CTCGCTTCCG GAGCGAAGGC GCAGGTAGAT CGTCCATCTA GGAACCGTAA CCTTGGCATT CCTGACGAGC GGACTGCTCG	GTTAGGTTTT TGCGACGCGA ACGCTGCGCT GCGGCATCGG CGCCGTAGCC GACGACCATC CTGCTGGTAG AAAGGCCGCG TTTCCGGCGC ATCACAAAAA TAGTGTTTTT TAAAGATACC	ATTCCATATA GGCTGGATGG CCGACCTACC GATGCCCGCG CTACGGGCGC AGGGACAGCT TCCCTGTCGA TTGCTGGCGT AACGACCGCA ACGACCGCA AGCTGCGAGT AGCTGCGAGT	ATAACTACTA CCTTCCCCAT GGAAGGGGTA TTGCAGGCCA AACGTCCGGT TCAAGGCCAG AGTTCCGGTC TTTTCCATAG AAAAGGTATC AGTCAGAGGT TCAGTCTCCA CCCTGGAAGGC	CAATTAATTC TATGATTCTT ATACTAAGAA TGCTGTCCAG ACGACAGGTC CAAAAGGCCA GTTTTCCGGT GCTCCGCCCC CGAGGCGGGG GCCGAAACCC CCGCTTTGGG TCCCTCGTGC
34601 34651 34701 34751 34801	TATAACCGAA AATTCGGATC TTAAGCCTAG CTCGCTTCCG GAGCGAAGGC GCAGGTAGAT CGTCCATCTA GGAACCGTAA CCTTGGCATT CCTGACGAGC GGACTGCTCG	GTTAGGTTTT TGCGACGCGA ACGCTGCGCT GCGGCATCGG CGCCGTAGCC GACGACCATC CTGCTGGTAG AAAGGCCGCG TTTCCGGCGC ATCACAAAAA TAGTGTTTTT TAAAGATACC	ATTCCATATA GGCTGGATGG CCGACCTACC GATGCCCGCG CTACGGGCGC AGGGACAGCT TCCCTGTCGA TTGCTGGCGT AACGACCGCA ACGACCGCA AGCTGCGAGT AGCTGCGAGT	ATAACTACTA CCTTCCCCAT GGAAGGGGTA TTGCAGGCCA AACGTCCGGT TCAAGGCCAG AGTTCCGGTC TTTTCCATAG AAAAGGTATC AGTCAGAGGT TCAGTCTCCA CCCTGGAAGGC	CAATTAATTC TATGATTCTT ATACTAAGAA TGCTGTCCAG ACGACAGGTC CAAAAGGCCA GTTTTCCGGT GCTCCGCCCC CGAGGCGGGG GGCGAAACCC CCGCTTTGGG
34601 34651 34701 34751 34801 34851	TATAACCGAA AATTCGGATC TTAAGCCTAG CTCGCTTCCG GAGCGAAGGC GCAGGTAGAT CGTCCATCTA CCTTGGCATT CCTGACGAGC GGACTGCTCG GACAGGACTA CTTGCCATCT CTGACGAGC GGACTGCTCG GACAGGACTA CTGTCCTGAT	GTTAGGTTTT TGCGACGCGA ACGCTGCGCT GCGGCATCGG CGCCGTAGCC GACGACCATC CTGCTGGTAG AAAGGCCGCG TTTCCGGCGC ATCACAAAAA TAGTGTTTT TAAAGATACC ATTTCTATGG	ATTCCATATA GGCTGGATGG CCGACCTACC GATGCCCGCG CTACGGCGCC AGGGACAGCT TCCCTGTCGA TTGCTGGCGT AACGACCGCA AGCTGCGAGT AGCTGCGAGT CCGCAAAGG CCGCTTACCG	ATAACTACTA CCTTCCCCAT GGAAGGGGTA TTGCAGGCCA AACGTCCGGT TCAAGGCCAG AGTTCCGTC TTTTCCATAG AAAAGGTATC AGTCAGAGGT TCAGTCTCCA CCCTGGAAGC GGGACCTTCG GATACCTGTC	CAATTAATTC TATGATTCTT ATACTAAGAA TGCTGTCCAG ACGACAGGTC CAAAAGGCCA GTTTTCCGGT GCTCCGCCCC CGAGGCGGGG GCGAAACCC CCGCTTTGGG TCCCTCGTGC ACGGAGCACC CGCCTTTCTC
34601 34651 34701 34751 34801 34851	TATAACCGAA AATTCGGATC TTAAGCCTAG CTCGCTTCCG GAGCGAAGGC GCAGGTAGAT CGTCCATCTA CCTTGGCATT CCTGACGAGC GGACTGCTCG GACAGGACTA CTTGCCATCT CTGACGAGC GGACTGCTCG GACAGGACTA CTGTCCTGAT	GTTAGGTTTT TGCGACGCGA ACGCTGCGCT GCGGCATCGG CGCCGTAGCC GACGACCATC CTGCTGGTAG AAAGGCCGCG TTTCCGGCGC ATCACAAAAA TAGTGTTTT TAAAGATACC ATTTCTATGG	ATTCCATATA GGCTGGATGG CCGACCTACC GATGCCCGCG CTACGGCGCC AGGGACAGCT TCCCTGTCGA TTGCTGGCGT AACGACCGCA AGCTGCGAGT AGCTGCGAGT CCGCAAAGG CCGCTTACCG	ATAACTACTA CCTTCCCCAT GGAAGGGGTA TTGCAGGCCA AACGTCCGGT TCAAGGCCAG AGTTCCGTC TTTTCCATAG AAAAGGTATC AGTCAGAGGT TCAGTCTCCA CCCTGGAAGC GGGACCTTCG GATACCTGTC	TATGATTCTT ATACTAAGAA TGCTGTCCAG ACGACAGGTC CAAAAGGCCA GTTTTCCGGT GCTCCGCCCC CGAGGCGGGG GCGAAACCC CCGCTTTGGG TCCCTCGTGC ACGGAGCCACG
34601 34651 34701 34751 34801 34851 34901	TATAACCGAA AATTCGGATC TTAAGCCTAG CTCGCTTCCG GAGCGAAGGC GCAGGTAGAT CGTCCATCTA CCTTGGCATT CCTGACGAGC GGACTGCTCG GACAGGACTA CTGTCCTGAT CTGTCCTGAT CTGTCCTGAT CTGTCCTGAT	GTTAGGTTTT TGCGACGCGA ACGCTGCGCT GCGGCATCGG CGCCGTAGCC GACGACCATC CTGCTGGTAG AAAGGCCGCG TTTCCGGCGC ATCACAAAAA TAGTGTTTT TAAAGATACC ATTTCTATGG TCCGACCCTG AGGCTGGGAC	ATTCCATATA GGCTGGATGG CCGACCTACC GATGCCCGCG CTACGGCGCC AGGGACAGCT TCCCTGTCGA TTGCTGGCGT AACGACCGCA AGCTGCGAGT AGCTGCGAGT CCGCAAAGG CCGCTTACCG GGCGAATGGC	ATAACTACTA CCTTCCCCAT GGAAGGGGTA TTGCAGGCCA AACGTCCGGT TCAAGGCCAG AGTTCCGGTC TTTTCCATAG AAAAGGTATC AGTCAGAGGT TCAGTCTCCA CCCTGGAAGC GGGACCTTCG GATACCTGTC CTATGGACAG	CAATTAATTC TATGATTCTT ATACTAAGAA TGCTGTCCAG ACGACAGGTC CAAAAGGCCA GTTTTCCGGT GCTCCGCCCC CGAGGCGGGG GCCAAACCC CCGCTTTGGG TCCCTCGTGC ACGGAGCACC CGCCTTTCTC CGCCCTTTCTC CCCCCAAAGAG
34601 34651 34701 34751 34801 34851 34901	TATAACCGAA AATTCGGATC TTAAGCCTAG CTCGCTTCCG GAGCGAAGGC GCAGGTAGAT CGTCCATCTA CCTTGGCATCT CCTGACGAGC GGACTGCTCG GACAGGACTA CTGTCCTGAT CTGTCCTGAT CTGTCCTGAT CCTCCTGGTAA CTGTCCTGAT CCTGTCCTGAT CCTCCTGGTAA	GTTAGGTTTT TGCGACGCGA ACGCTGCGCT GCGGCATCGG CGCCGTAGCC GACGACCATC CTGCTGGTAG AAAGGCCGCG ATCACAAAAA TAGTGTTTT TAAAGATACC ATTTCTATGG TCCGACCCTG AGGCTGGCAC	ATTCCATATA GGCTGGATGG CCGACCTACC GATGCCCGCG CTACGGCGCC AGGGACAGCT TCCCTGTCGA TTGCTGGCGT AACGACCGCA AGCTGCGAGT AGCGCTTACCG CCGCTTACCG GGCGAATGGC TTCTCATAGC	ATAACTACTA CCTTCCCCAT GGAAGGGGTA TTGCAGGCCA AACGTCCGGT TCAAGGCCAG AGTTCCGGTC TTTTCCATAG AAAAGGTATC AGTCAGAGGT TCAGTCTCCA CCCTGGAAGC GGGACCTTCG GATACCTGTC CTATGGACAG TCACGCTGTA	TATGATTCTT ATACTAAGAA TGCTGTCCAG ACGACAGGTC CAAAAGGCCA GTTTTCCGGT GCTCCGCCCC CGAGGCGGGG GCCAAACCC CCGCTTTGGG TCCCTCGTGC ACGGAGCACG CGCCTTTCTC CGCGAAAGAG GGCAAACGC
34601 34651 34701 34751 34801 34851 34901	TATAACCGAA AATTCGGATC TTAAGCCTAG CTCGCTTCCG GAGCGAAGGC GCAGGTAGAT CGTCCATCTA CCTTGGCATT CCTGACGAGC GGACTGCTCG GACAGGACTA CTGTCCTGAT CTGTCCTGAT CTGTCCTGAT CCTCTCGGAT CCTCCTGT	GTTAGGTTTT TGCGACGCGA ACGCTGCGCT GCGGCATCGG CGCCGTAGCC GACGACCATC CTGCTGGTAG AAAGGCCGCG ATCACAAAAA TAGTGTTTTT TAAAGATACC ATTTCTATGG TCCGACCCTG AGGCTGGCGCT CGCACCGCGA	ATTCCATATA GGCTGGATGG CCGACCTACC GATGCCCGCG CTACGGGCGC AGGGACAGCT TCCCTGTCGA TTGCTGGCGT AACGACCGCA AGCTGCGAGT AGCGCTTACCG CCGCTTACCG GGCGAATGGC TTCTCATAGC AAGAGTATCG	ATAACTACTA CCTTCCCCAT GGAAGGGGTA TTGCAGGCCA AACGTCCGGT TCAAGGCCAG AGTTCCGGTC TTTTCCATAG AAAAGGTATC AGTCAGAGGT TCAGTCTCCA CCCTGGAAGC GGGACCTTCG GATACCTGTC CTATGGACAG TCACGCTGTA AGTCCGACAG	TATGATTCTT ATACTAAGAA TGCTGTCCAG ACGACAGGTC CAAAAGGCCA GTTTTCCGGT GCTCCGCCCC CGAGGCGGGG GCGAAACCC CCGCTTTGGG TCCCTCGTGC AGGGAGCACG CGCCTTTCTC GCGGAAAGAG GGTATCTCAG GCTATCTCAG CCATAGAGTC
34601 34651 34701 34751 34801 34851 34901	TATAACCGAA AATTCGGATC TTAAGCCTAG CTCGCTTCCG GAGCGAAGGC GCAGGTAGAT CGTCCATCTA CCTTGGCATA CCTTGGCATT CCTGACGAGC GGACTGCTCG GACAGGACTA CTGTCCTGAT CTGTCCTGAT CCTCTCGGAT CTTCCTGT CGAGAGGACA CCTTCGGGAA CCTTCGGGAA CCTTCGGGAA CCTTCGGGAA TTCGGTGTAG	GTTAGGTTTT TGCGACGCGA ACGCTGCGCT GCGGCATCGG CGCCGTAGCC GACGACCATC CTGCTGGTAG AAAGGCCGCG ATCACAAAAA TAGTGTTTT TAAAGATACC ATTTCTATGG TCCGACCCTG AGGCTGGCGCT CGCACCGCGA	ATTCCATATA GGCTGGATGG CCGACCTACC GATGCCCGCG CTACGGGCGC AGGGACAGCT TCCCTGTCGA TTGCTGGCGT AACGACCGCA AGCTGCGAGT CCGCAAAGG CCGCTTACCG GGCGAATGGC TTCTCATAGC AAGAGTATCG CCAAGCTGGG CCAAGCTGGG	ATAACTACTA CCTTCCCCAT GGAAGGGGTA TTGCAGGCCA AACGTCCGGT TCAAGGCCAG AGTTCCGGTC TTTTCCATAG AAAAGGTATC AGTCAGAGGT TCAGTCTCCA CCCTGGAAGC GGGACCTTCG GATACCTGTC CTATGGACAG TCACGCTGTA AGTCCGACAT CCTGTGCACAC	TATGATTCTT ATACTAAGAA TGCTGTCCAG ACGACAGGTC CAAAAGGCCA GTTTTCCGGT GCTCGCCCC CGAGGCGGGG GCGAAACCC CCGCTTTGGG TCCCTCGTGC AGGGAGCACG CGCCTTTCTC GCGGAAAGAG GGTATCTCAG CCATAGAGTC
34601 34651 34701 34751 34801 34851 34901	TATAACCGAA AATTCGGATC TTAAGCCTAG CTCGCTTCCG GAGCGAAGGC GCAGGTAGAT CGTCCATCTA CCTTGGCATA CCTTGGCATT CCTGACGAGC GGACTGCTCG GACAGGACTA CTGTCCTGAT CTGTCCTGAT CCTCTCGGAT CTTCCTGT CGAGAGGACA CCTTCGGGAA CCTTCGGGAA CCTTCGGGAA CCTTCGGGAA TTCGGTGTAG	GTTAGGTTTT TGCGACGCGA ACGCTGCGCT GCGGCATCGG CGCCGTAGCC GACGACCATC CTGCTGGTAG AAAGGCCGCG ATCACAAAAA TAGTGTTTT TAAAGATACC ATTTCTATGG TCCGACCCTG AGGCTGGCGCT CGCACCGCGA	ATTCCATATA GGCTGGATGG CCGACCTACC GATGCCCGCG CTACGGGCGC AGGGACAGCT TCCCTGTCGA TTGCTGGCGT AACGACCGCA AGCTGCGAGT CCGCAAAGG CCGCTTACCG GGCGAATGGC TTCTCATAGC AAGAGTATCG CCAAGCTGGG CCAAGCTGGG	ATAACTACTA CCTTCCCCAT GGAAGGGGTA TTGCAGGCCA AACGTCCGGT TCAAGGCCAG AGTTCCGGTC TTTTCCATAG AAAAGGTATC AGTCAGAGGT TCAGTCTCCA CCCTGGAAGC GGGACCTTCG GATACCTGTC CTATGGACAG TCACGCTGTA AGTCCGACAT CCTGTGCACAC	TATGATTCTT ATACTAAGAA TGCTGTCCAG ACGACAGGTC CAAAAGGCCA GTTTTCCGGT GCTCCGCCCC CGAGGCGGGG GCGAAACCC CCGCTTTGGG TCCCTCGTGC AGGGAGCACG CGCCTTTCTC GCGGAAAGAG GGTATCTCAG GCTATCTCAG CCATAGAGTC

Figure 27 AK

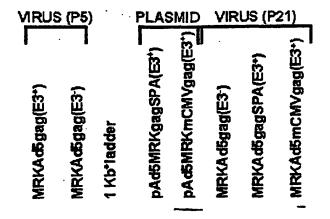
35051		CCGCTGCGCC GGCGACGCGG	 	
35101	CCGGTAAGAC			
	•	TGCTGAATAG		
35151		AGGTATGTAG TCCATACATC		
35201		CTACACTAGA GATGTGATCT	 	
35251	AAGCCAGTTA TTCGGTCAAT	CCTTCGGAAA GGAAGCCTTT	 	
35301		GGTAGCGGTG CCATCGCCAC		
35351		AGGATCTCAA TCCTAGAGTT		
35401	•	GGAACGAAAA CCTTGCTTTT		
35451		ATCTTCACCT TAGAAGTGGA	 	
35501		TGGTCTGACA ACCAGACTGT		
35 551		CTGTCTATTT GACAGATAAA	 	
35601		CTACGATACG GATGCTATGC		-
35651		CGAGACCCAC GCTCTGGGTG	 	
35701		CGGAAGGGCC GCCTTCCCGG		
35751	GCCTCCATCC CGGAGGTAGG	AGTCTATTAA TCAGATAATT		
35801	GCCAGTTAAT CGGTCAATTA	AGTTTGCGCA TCAAACGCGT		
35851	TGTCACGCTC ACAGTGCGAG	GTCGTTTGGT CAGCAAACCA		
35901	TCAAGGCGAG AGTTCCGCTC	TTACATGATC AATGTACTAG		
35951	CTTCGGTCCT GAAGCCAGGA	CCGATCGTTG GGCTAGCAAC		

Figure 2 7AL

36001	TCATGGTTAT	AGCACTG	CATAATTCTC	TTACTGTCAT	GCCATCLTA
	AGTACCAATA	CCGTCGTGAC	GTATTAAGAG	AATGACAGTA	CGGTAGGCAT
36051	AGATGCTTTT	CTGTGACTGG	TGAGTACTCA	ACCAAGTCAT	TCTGAGAATA
	TCTACGAAAA	GACACTGACC	ACTCATGAGT	TGGTTCAGTA	AGACTCTTAT
36101				GGCGTCAACA	
	CACATACGCC	GCTGGCTCAA	CGAGAACGGG	CCGCAGTTGT	GCCCTATTAT
36151				TCATCATTGG	
	GGCGCGGTGT	ATCGTCTTGA	AATTTTCACG	AGTAGTAACC	TTTTGCAAGA
36201				CTGTTGAGAT	
	AGCCCCGCTT	TTGAGAGTTC	CTAGAATGGC	GACAACTCTA	GGTCAAGCTA
36251	GTAACCCACT	CGTGCACCCA	ACTGATCTTC	AGCATCTTTT	ACTTTCACCA
	CATTGGGTGA	GCACGTGGGT	TGACTAGAAG	TCGTAGAAAA	TGAAAGTGGT
36301	GCGTTTCTGG				
	CGCAAAGACC	CACTCGTTTT	TGTCCTTCCG	TTTTACGGCG	TTTTTTCCCT
36351	ATAAGGGCGA	CACGGAAATG	TTGAATACTC	ATACTCTTCC	TTTTTCAATA
	TATTCCCGCT	GTGCCTTTAC	AACTTATGAG	TATGAGAAGG	AAAAAGTTAT
36401				CATGAGCGGA	
	AATAACTTCG	TAAATAGTCC	CAATAACAGA	GTACTCGCCT	ATGTATAAAC
36451	AATGTATTTA	GAAAAATAAA	CAAATAGGGG	TTCCGCGCAC	ATTTCCCCGA
	TTACATAAAT	CTTTTTATTT	GTTTATCCCC	AAGGCGCGTG	TAAAGGGGCT
36501	AAAGTGCCAC	CTGACGTCTA	AGAAACCATT	ATTATCATGA	CATTAACCTA
	TTTCACGGTG	GACTGCAGAT	TCTTTGGTAA	TAATAGTACT	GTAATTGGAT
36551	TAAAAATAGG	CGTATCACGA	GGCCCTTTCG	TCTTCAAGAA	TTGGATCCGA
	ATTTTTATCC	GCATAGTGCT	CCGGGAAAGC	AGAAGTTCTT	AACCTAGGCT
		PacI			
36601	ATTCTTAATT	ΤΟΤΤΑΑΤΤΑΑ	(SEO ID NO	: 34)	
20001			(CEO TO NO		

TAAGAATTAA AGAATTAATT (SEQ ID NO:35)

Figure 27AM



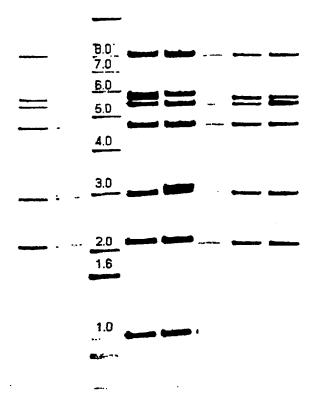


FIGURE 28

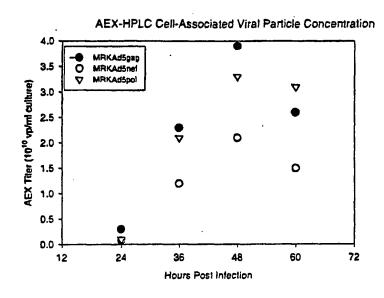


FIGURE 29A

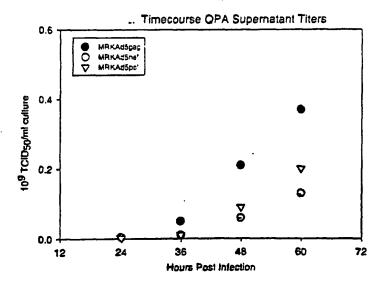


FIGURE 29B

atg Met 1	gat Asp	gca Ala	atg Met	aag Lys 5	aga Arg	Gly	ctc Leu	tgc Cys	tgt Cys 10	gtg Val	ctg Leu	ctg Leu	ctg Leu	tgt Cys 15	gga Gly	48
	gtc Val															96
gag Glu	ctg Leu	gag Glu 35	agg Arg	ttt Phe	gct Ala	gtg Val	aac Asn 40	cct Pro	ggc Gly	ctg Leu	ctg Leu	gag Glu 45	acc Thr	tct Ser	gag Glu	144
Gly ggg	tgc Cys 50	agg Arg	cag Gln	atc Ile	ctg Leu	ggc Gly 55	cag Gln	ctc Leu	cag Gln	ccc Pro	tcc Ser 60	ctg Leu	caa Gln	aca Thr	ggc Gly	192
tct Ser 65	gag Glu	gag Glu	ctg Leu	agg Arg	tcc Ser 70	ctg Leu	tac Tyr	aac Asn	aca Thr	gtg Val 75	gct Ala	acc Thr	ctg Leu	tac Tyr	tgt Cys 80	240
gtg Val	cac His	cag Gln	aag Lys	att Ile 85	gat Asp	gtg Val	aag Lys	gac Asp	acc Thr 90	aag Lys	gag Glu	gcc Ala	ctg Leu	gag Glu 95	aag Lys	288
att Ile	gag Glu	gag Glu	gag Glu 100	cag Gln	aac Asn	aag Lys	tcc Ser	aag Lys 105	aag Lys	aag Lys	gcc Ala	cag Gln	cag Gln 110	gct Ala	gct Ala	336
gct Ala	ggc Gly	aca Thr 115	ggc Gly	aac Asn	tcc Ser	agc Ser	cag Gln 120	gtg Val	tcc Ser	cag Gln	aac Asn	tac Tyr 125	ccc Pro	att Ile	gtg Val	384
cag Gln	aac Asn 130	ctc Leu	cag Gln	ggc	cag Gln	atg Met 135	gtg Val	cac His	cag Gln	gcc Ala	atc Ile 140	tcc Ser	ccc Pro	cgg Arg	acc Thr	432
ctg Leu 145	aat Asn	gcc Ala	tgg Trp	gtg Val	aag Lys 150	gtg Val	gtg Val	gag Glu	gag Glu	aag Lys 155	gcc Ala	ttc Phe	tcc Ser	cct Pro	gag Glu 160	480
gtg Val	atc Ile	ccc Pro	atg Met	ttc Phe 165	tct Ser	gcc Ala	ctg Leu	tct Ser	gag Glu 170	ggt Gly	gcc Ala	acc Thr	ccc Pro	cag Gln 175	gac Asp	528
ctg Leu	aac Asn	acc Thr	atg Met 180	ctg Leu	aac Asn	aca Thr	gtg Val	ggg Gly 185	ggc Gly	cat His	cag Gln	gct Ala	gcc Ala 190	atg Met	cag Gln	576
atg Met	ctg Leu	aag Lys 195	gag Glu	acc Thr	atc Ile	aat Asn	gag Glu 200	gag Glu	gct Ala	gct Ala	gag Glu	tgg Trp 205	gac Asp	agg Arg	ctg Leu	624
cat His	cct Pro 210	gtg Val	cac His	gct Ala	ggc	ccc Pro 215	att Ile	gcc Ala	ccc Pro	ggc Gly	cag Gln 220	atg Met	agg Arg	gag Glu	ccc Pro	672
agg Arg 225	ggc Gly	tct Ser	gac Asp	att Ile	gct Ala 230	ggc Gly	acc Thr	acc Thr	tcc Ser	acc Thr 235	ctc Leu	cag Gln	gag Glu	cag Gln	att Ile 240	720
ggc Gly	tgg Trp	atg Met	acc Thr	aac Asn 245	aac Asn	ccc Pro	ccc Pro	atc Ile	cct Pro 250	gtg Val	ggg Gly	gaa Glu	atc Ile	tac Tyr 255	aag Lys	768

Figure 30'A"

agg Arg	tgg Trp	atc Ile	atc Ile 260	ctg Leu	ggc Gly	ctg Leu	aac Asn	aag Lys 265	att Ile	gtg Val	agg Arg	atg Met	tac Tyr 270	tcc Ser	CCC Pro	816
acc Thr	tcc Ser	atc Ile 275	ctg Leu	gac Asp	atc Ile	agg Arg	cag Gln 280	ggc Gly	ccc Pro	aag Lys	gag Glu	ccc Pro 285	ttc Phe	agg Arg	gac Asp	864
tat Tyr	gtg Val 290	gac Asp	agg Arg	ttc Phe	tac Tyr	aag Lys 295	acc Thr	ctg Leu	agg Arg	gct Ala	gag Glu 300	cag Gln	gcc Ala	tcc Ser	cag Gln	912
gag Glu 305	gtg Val	aag Lys	aac Asn	tgg Trp	atg Met 310	aca Thr	gag Glu	acc Thr	ctg Leu	ctg Leu 315	gtg Val	cag Gln	aat Asn	gcc Ala	aac Asn 320	960
cct Pro	gac Asp	tgc Cys	aag Lys	acc Thr 325	atc Ile	ctg Leu	aag Lys	gcc Ala	ctg Leu 330	ggc	ect Pro	gct Ala	gcc Ala	acc Thr 335	ctg Leu	1008
gag Glu	gag Glu	atg Met	atg Met 340	aca Thr	gcc Ala	Çys Çys	cag Gln	ggg Gly 345	gtg Val	GJA aaa	ggc Gly	cct Pro	ggt Gly 350	cac His	aag Lys	1056
gcc Ala	agg Arg	gtg Val 355	ctg Leu	gct Ala	gag Glu	gcc Ala	atg Met 360	tcc Ser	cag Gln	gtg Val	acc Thr	aac Asn 365	tcc Ser	gcc Ala	acc Thr	1104
atc Ile	atg Met 370	atg Met	cag Gln	agg Arg	ggc Gly	aac Asn 375	ttc Phe	agg Arg	aac Asn	cag Gln	agg Arg 380	aag Lys	aca Thr	gtg Val	aag Lys	1152
tgc Cys 385	Phe	aac Asn	tgt Cys	ggc Gly	aag Lys 390	gtg Val	ggc	cac His	att Ile	gcc Ala 395	aag Lys	aac Asn	tgt Cys	agg Arg	gcc Ala 400	1200
ccc Pro	agg Arg	aag Lys	aag Lys	ggc Gly 405	tgc Cys	tgg Trp	aag Lys	tgt Cys	ggc Gly 410	aag Lys	gag Glu	ggc	cac His	cag Gln 415	atg Met	1248
aag Lys	gac Asp	tgc Cys	aat Asn 420	gag Glu	agg Arg	cag Gln	gcc Ala	aac Asn 425	ttc Phe	ctg Leu	Gly ggc	aaa Lys	atc Ile 430	tgg Trp	ccc Pro	1296
tcc Ser	cac His	aag Lys 435	ggc Gly	agg Arg	cct Pro	ggc Gly	aac Asn 440	ttc Phe	ctc Leu	cag Gln	tcc Ser	agg Arg 445	cct Pro	gag Glu	ccc Pro	1344
aca Thr	gcc Ala 450	cct Pro	ccc Pro	gag Glu	gag Glu	tcc Ser 455	ttc Phe	agg Arg	ttť Phe	GJĀ āāā	gag Glu 460	gag Glu	aag Lys	acc Thr	acc Thr	1392
ccc Pro 465	agc Ser	cag Gln	aag Lys	cag Gln	gag Glu 470	ccc Pro	att Ile	gac Asp	aag Lys	gag Glu 475	ctg Leu	tac Tyr	ccc Pro	ctg Leu	gcc Ala 480	1440
tcc Ser	ctg Leu	agg Arg	tcc Ser	ctg Leu 485	ttt Phe	ggc Gly	aac Asn	gac Asp	ccc Pro 490	tcc Ser	tcc Ser	cag Gln	taa *	(SII	NO:36)	1482

Figure 30 B

Figure 31

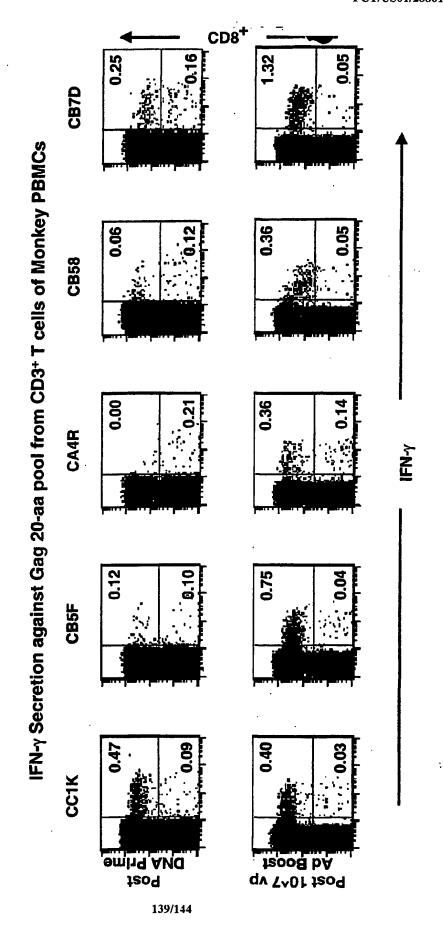


FIGURE 32

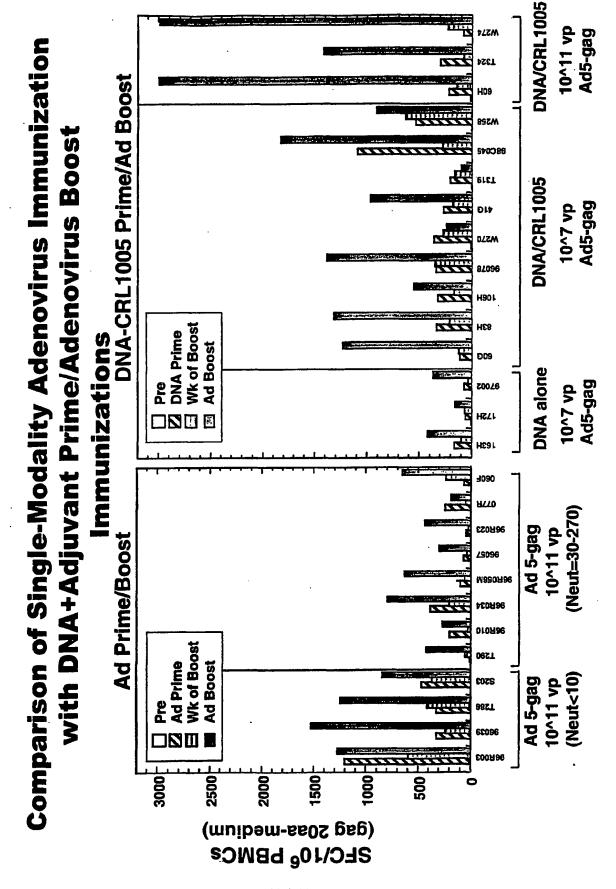


FIGURE 33A

ATGGGTGCTA	GGGCTTCTGT	GCTGTCTGGT	GGTGAGCTGG	ACAAGTGGGA	GAAGATCAGG
CTGAGGCCTG	GTGGCAAGAA	GAAGTACAAG	CTAAAGCACA	TTGTGTGGGC	CTCCAGGGAG
CTGGAGAGGT	TTGCTGTGAA	CCCTGGCCTG	CTGGAGACCT	CTGAGGGGTG	CAGGCAGATC
CTGGGCCAGC	TCCAGCCCTC	CCTGCAAACA	GGCTCTGAGG	AGCTGAGGTC	CCTGTACAAC
ACAGTGGCTA	CCCTGTACTG	TGTGCACCAG	AAGATTGATG	TGAAGGACAC	CAAGGAGGCC
CTGGAGAAGA	TTGAGGAGGA	GCAGAACAAG	TCCAAGAAGA	AGGCCCAGCA	GGCTGCTGCT
GGCACAGGCA	ACTCCAGCCA	GGTGTCCCAG	AACTACCCCA	TTGTGCAGAA	CCTCCAGGGC
CAGATGGTGC	ACCAGGCCAT	CTCCCCCGG	ACCCTGAATG	CCTGGGTGAA	GGTGGTGGAG
GAGAAGGCCT	TCTCCCCTGA	GGTGATCCCC	ATGTTCTCTG	CCCTGTCTGA	GGGTGCCACC
CCCCAGGACC	TGAACACCAT	GCTGAACACA	GTGGGGGGCC	ATCAGGCTGC	CATGCAGATG
CTGAAGGAGA	CCATCAATGA	GGAGGCTGCT	GAGTGGGACA	GGCTGCATCC	TGTGCACGCT
GGCCCCATTG	CCCCGGCCA	GATGAGGGAG	CCCAGGGGCT	CTGACATTGC	TGGCACCACC
TCCACCCTCC	AGGAGCAGAT	TGGCTGGATG	ACCAACAACC	CCCCCATCCC	TGTGGGGGAA
ATCTACAAGA	${\tt GGTGGATCAT}$	CCTGGGCCTG	AACAAGATTG	TGAGGATGTA	CTCCCCCACC
TCCATCCTGG	ACATCAGGCA	GGGCCCCAAG	GAGCCCTTCA	GGGACTATGT	GGACAGGTTC
TACAAGACCC	${\tt TGAGGGCTGA}$	GCAGGCCTCC	CAGGAGGTGA	AGAACTGGAT	GACAGAGACC
CTGCTGGTGC	AGAATGCCAA	CCCTGACTGC	AAGACCATCC	TGAAGGCCCT	GGGCCCTGCT
${\tt GCCACCCTGG}$	AGGAGATGAT	GACAGCCTGC	CAGGGGGTGG	GGGCCCTGG	TCACAAGGCC
AGGGTGCTGG	CTGAGGCCAT	GTCCCAGGTG	ACCAACTCCG	CCACCATCAT	GATGCAGAGG
GGCAACTTCA	GGAACCAGAG	GAAGACAGTG	AAGTGCTTCA	ACTGTGGCAA	GGTGGGCCAC
ATTGCCAAGA	ACTGTAGGGC	CCCCAGGAAG	AAGGGCTGCT	GGAAGTGTGG	CAAGGAGGGC
CACCAGATGA	AGGACTGCAA	TGAGAGGCAG	GCCAACTTCC	TGGGCAAAAT	CTGGCCCTCC
CACAAGGGCA	GGCCTGGCAA	CTTCCTCCAG	TCCAGGCCTG	AGCCCACAGC	CCCTCCCGAG
GAGTCCTTCA	GGTTTGGGGA	GGAGAAGACC	ACCCCCAGCC	AGAAGCAGGA	GCCCATTGAC
AAGGAGCTGT	ACCCCCTGGC	CTCCCTGAGG	TCCCTGTTTG	GCAACGACCC	CTCCTCCCAG
ATGGCTCCCA	TCTCCCCCAT	TGAGACTGTG	CCTGTGAAGC	TGAAGCCTGG	CATGGATGGC
CCCAAGGTGA	AGCAGTGGCC	CCTGACTGAG	GAGAAGATCA	AGGCCCTGGT	GGAAATCTGC
ACTGAGATGG	AGAAGGAGGG	CAAAATCTCC	AAGATTGGCC	CCGAGAACCC	CTACAACACC
CCTGTGTTTG	CCATCAAGAA	GAAGGACTCC	ACCAAGTGGA	GGAAGCTGGT	GGACTTCAGG
GAGCTGAACA	AGAGGACCCA	GGACTTCTGG	GAGGTGCAGC	TGGGCATCCC	CCACCCCGCT
GGCCTGAAGA	AGAAGAAGTC	TGTGACTGTG	CTGGCTGTGG	GGGATGCCTA	CTTCTCTGTG
CCCCTGGATG	AGGACTTCAG	GAAGTACACT	GCCTTCACCA	TCCCCTCCAT	CAACAATGAG
ACCCCTGGCA	TCAGGTACCA	GTACAATGTG	CTGCCCCAGG	GCTGGAAGGG	CTCCCCTGCC
ATCTTCCAGT	CCTCCATGAC	CAAGATCCTG	GAGCCCTTCA	GGAAGCAGAA	CCCTGACATT
GTGATCTACC	AGTACATGGC	TGCCCTGTAT	GTGGGCTCTG	ACCTGGAGAT	TGGGCAGCAC
AGGACCAAGA	TTGAGGAGCT	GAGGCAGCAC	CTGCTGAGGT	GGGGCCTGAC	CACCCCTGAC
	AGAAGGAGCC				
TGGACTGTGC	AGCCCATTGT	GCTGCCTGAG	AAGGACTCCT	GGACTGTGAA	TGACATCCAG
AAGCTGGTGG	GCAAGCTGAA	CTGGGCCTCC	CAAATCTACC	CTGGCATCAA	GGTGAGGCAG
	TGCTGAGGGG				
GCTGAGCTGG	AGCTGGCTGA	GAACAGGGAG	ATCCTGAAGG	AGCCTGTGCA	TGGGGTGTAC

FIGURE 33B

TATGACCCCT	CCAAGGACCT	GATTGCTGAG	ATCCAGAAGC	AGGGCCAGGG	CCAGTGGACC
TACCAAATCT	ACCAGGAGCC	CTTCAAGAAC	CTGAAGACTG	GCAAGTATGC	CAGGATGAGG
GGGGCCCACA	CCAATGATGT	GAAGCAGCTG	ACTGAGGCTG	TGCAGAAGAT	CACCACTGAG
TCCATTGTGA	TCTGGGGCAA	GACCCCCAAG	TTCAAGCTGC	CCATCCAGAA	GGAGACCTGG
GAGACCTGGT	GGACTGAGTA	CTGGCAGGCC	ACCTGGATCC	CTGAGTGGGA	GTTTGTGAAC
ACCCCCCCC	TGGTGAAGCT	GTGGTACCAG	CTGGAGAAGG	${\tt AGCCCATTGT}$	GGGGGCTGAG
ACCTTCTATG	TGGCTGGGGC	TGCCAACAGG	GAGACCAAGC	TGGGCAAGGC	TGGCTATGTG
ACCAACAGGG	GCAGGCAGAA	GGTGGTGACC	CTGACTGACA	CCACCAACCA	GAAGACTGCC
CTCCAGGCCA	TCTACCTGGC	CCTCCAGGAC	TCTGGCCTGG	AGGTGAACAT	TGTGACTGCC
TCCCAGTATG	CCCTGGGCAT	CATCCAGGCC	CAGCCTGATC	AGTCTGAGTC	TGAGCTGGTG
AACCAGATCA	TTGAGCAGCT	GATCAAGAAG	GAGAAGGTGT	ACCTGGCCTG	GGTGCCTGCC
CACAAGGGCA	TTGGGGGCAA	TGAGCAGGTG	GACAAGCTGG	TGTCTGCTGG	CATCAGGAAG
GTGCTGTTCC	TGGATGGCAT	TGACAAGGCC	CAGGATGAGC	ATGAGAAGTA	CCACTCCAAC
TGGAGGGCTA	TGGCCTCTGA	${\tt CTTCAACCTG}$	CCCCTGTGG	${\tt TGGCTAAGGA}$	GATTGTGGCC
TCCTGTGACA	AGTGCCAGCT	GAAGGGGGAG	GCCATGCATG	${\tt GGCAGGTGGA}$	CTGCTCCCCT
GGCATCTGGC	AGCTGGCCTG	CACCCACCTG	GAGGGCAAGG	TGATCCTGGT	GGCTGTGCAT
GTGGCCTCCG	GCTACATTGA	GGCTGAGGTG	ATCCCTGCTG	AGACAGGCCA	GGAGACTGCC
TACTTCCTGC	TGAAGCTGGC	TGGCAGGTGG	CCTGTGAAGA	CCATCCACAC	TGCCAATGGC
TCCAACTTCA	CTGGGGCCAC	AGTGAGGGCT	GCCTGCTGGT	GGGCTGGCAT	CAAGCAGGAG
TTTGGCATCC	CCTACAACCC	CCAGTCCCAG	GGGGTGGTGG	CCTCCATGAA	CAAGGAGCTG
AAGAAGATCA	${\tt TTGGGCAGGT}$	GAGGGACCAG	GCTGAGCACC	TGAAGACAGC	TGTGCAGATG
GCTGTGTTCA	TCCACAACTT	CAAGAGGAAG	GGGGGCATCG	GGGGCTACTC	CGCTGGGGAG
AGGATTGTGG	ACATCATTGC	CACAGACATC	CAGACCAAGG	AGCTCCAGAA	GCAGATCACC
AAGATCCAGA	ACTTCAGGGT	GTACTACAGG	GACTCCAGGA	ACCCCCTGTG	GAAGGGCCCT
GCCAAGCTGC	TGTGGAAGGG	GGAGGGGCT	GTGGTGATCC	AGGACAACTC	TGACATCAAG
GTGGTGCCCA	GGAGGAAGGC	CAAGATCATC	AGGGACTATG	GCAAGCAGAT	GGCTGGGGAT
GACTGTGTGG	CCTCCAGGCA	GGATGAGGAC	TAA		
SEQ ID NO:	38				

142/144

FIGURE 34A

Met Gly Ala Arg Ala Ser Val Leu Ser Gly Gly Glu Leu Asp Lys Trp Glu Lys Ile Arg Leu Arg Pro Gly Gly Lys Lys Lys Tyr Lys Leu Lys His Ile Val Trp Ala Ser Arg Glu Leu Glu Arg Phe Ala Val Asn Pro Gly Leu Leu Glu Thr Ser Glu Gly Cys Arg Gln Ile Leu Gly Gln Leu Gln Pro Ser Leu Gln Thr Gly Ser Glu Glu Leu Arg Ser Leu Tyr Asn Thr Val Ala Thr Leu Tyr Cys Val His Gln Lys Ile Asp Val Lys Asp Thr Lys Glu Ala Leu Glu Lys Ile Glu Glu Glu Glu Asn Lys Ser Lys Lys Lys Ala Gln Gln Ala Ala Gly Thr Gly Asn Ser Ser Gln Val Ser Gln Asn Tyr Pro Ile Val Gln Asn Leu Gln Gly Gln Met Val His Gln Ala Ile Ser Pro Arg Thr Leu Asn Ala Trp Val Lys Val Val Glu Glu Lys Ala Phe Ser Pro Glu Val Ile Pro Met Phe Ser Ala Leu Ser Glu Gly Ala Thr Pro Gln Asp Leu Asn Thr Met Leu Asn Thr Val Gly Gly His Gln Ala Ala Met Gln Met Leu Lys Glu Thr Ile Asn Glu Glu Ala Ala Glu Trp Asp Arg Leu His Pro Val His Ala Gly Pro Ile Ala Pro Gly Gln Met Arg Glu Pro Arg Gly Ser Asp Ile Ala Gly Thr Thr Ser Thr Leu Gln Glu Gln Ile Gly Trp Met Thr Asn Asn Pro Pro Ile Pro Val Gly Glu Ile Tyr Lys Arg Trp Ile Ile Leu Gly Leu Asn Lys Ile Val Arg Met Tyr Ser Pro Thr Ser Ile Leu Asp Ile Arg Gln Gly Pro Lys Glu Pro Phe Arg Asp Tyr Val Asp Arg Phe Tyr Lys Thr Leu Arg Ala Glu Gln Ala Ser Gln Glu Val Lys Asn Trp Met Thr Glu Thr Leu Leu Val Gln Asn Ala Asn Pro Asp Cys Lys Thr Ile Leu Lys Ala Leu Gly Pro Ala Ala Thr Leu Glu Glu Met Met Thr Ala Cys Gln Gly Val Gly Gly Pro Gly His Lys Ala Arg Val Leu Ala Glu Ala Met Ser Gln Val Thr Asn Ser Ala Thr Ile Met Met Gln Arg Gly Asn Phe Arg Asn Gln Arg Lys Thr Val Lys Cys Phe Asn Cys Gly Lys Val Gly His Ile Ala Lys Asn Cys Arg Ala Pro Arg Lys Lys Gly Cys Trp Lys Cys Gly Lys Glu Gly His Gln Met Lys Asp Cys Asn Glu Arg Gln Ala Asn Phe Leu Gly Lys Ile Trp Pro Ser His Lys Gly Arg Pro Gly Asn Phe Leu Gln Ser Arg Pro Glu Pro Thr Ala Pro Pro Glu Glu Ser Phe Arg Phe Gly Glu Glu Lys Thr Thr Pro Ser Gln Lys Gln Glu Pro Ile Asp Lys Glu Leu Tyr Pro Leu Ala Ser Leu Arg Ser Leu Phe Gly Asn Asp Pro Ser Ser Gln Met Ala Pro Ile Ser Pro Ile Glu Thr Val Pro Val Lys Leu Lys Pro Gly Met Asp Gly Pro Lys Val Lys Gln Trp Pro Leu Thr Glu Glu Lys Ile Lys Ala Leu Val Glu Ile Cys Thr Glu Met Glu Lys Glu Gly Lys Ile Ser Lys Ile Gly Pro Glu Asn Pro Tyr Asn Thr Pro Val Phe Ala Ile Lys Lys Lys Asp Ser Thr Lys Trp Arg Lys Leu Val Asp Phe Arg Glu Leu Asn Lys Arg Thr Gln Asp Phe Trp Glu Val Gln Leu Gly Ile Pro His Pro Ala Gly Leu Lys Lys Lys Ser Val Thr Val Leu Ala Val Gly Asp Ala Tyr Phe Ser Val Pro Leu Asp Glu Asp Phe Arg Lys Tyr Thr Ala Phe Thr Ile Pro Ser Ile Asn Asn Glu Thr Pro Gly Ile Arg Tyr Gln Tyr Asn Val Leu Pro Gln Gly Trp Lys Gly Ser Pro Ala Ile Phe Gln Ser Ser Met Thr Lys Ile Leu Glu Pro Phe Arg Lys Gln Asn Pro Asp Ile Val Ile Tyr Gln Tyr Met Ala Ala Leu Tyr Val Gly Ser Asp Leu Glu Ile Gly Gln His Arg Thr Lys Ile Glu Glu Leu Arg Gln His Leu Leu Arg Trp Gly Leu Thr Thr Pro Asp Lys Lys His Gln Lys Glu Pro Pro Phe Leu Trp Met Gly Tyr Glu Leu His Pro

FIGURE 34B

Asp Lys Trp Thr Val Gln Pro Ile Val Leu Pro Glu Lys Asp Ser Trp Thr Val Asn Asp Ile Gln Lys Leu Val Gly Lys Leu Asn Trp Ala Ser Gln Ile Tyr Pro Gly Ile Lys Val Arg Gln Leu Cys Lys Leu Leu Arg Gly Thr Lys Ala Leu Thr Glu Val Ile Pro Leu Thr Glu Glu Ala Glu Leu Glu Leu Ala Glu Asn Arg Glu Ile Leu Lys Glu Pro Val His Gly Val Tyr Tyr Asp Pro Ser Lys Asp Leu Ile Ala Glu Ile Gln Lys Gln Gly Gln Trp Thr Tyr Gln Ile Tyr Gln Glu Pro Phe Lys Asn Leu Lys Thr Gly Lys Tyr Ala Arg Met Arg Gly Ala His Thr Asn Asp Val Lys Gln Leu Thr Glu Ala Val Gln Lys Ile Thr Thr Glu Ser Ile Val Ile Trp Gly Lys Thr Pro Lys Phe Lys Leu Pro Ile Gln Lys Glu Thr Trp Glu Thr Trp Trp Thr Glu Tyr Trp Gln Ala Thr Trp Ile Pro Glu Trp Glu Phe Val Asn Thr Pro Pro Leu Val Lys Leu Trp Tyr Gln Leu Glu Lys Glu Pro Ile Val Gly Ala Glu Thr Phe Tyr Val Ala Gly Ala Ala Asn Arg Glu Thr Lys Leu Gly Lys Ala Gly Tyr Val Thr Asn Arg Gly Arg Gln Lys Val Val Thr Leu Thr Asp Thr Thr Asn Gln Lys Thr Ala Leu Gln Ala Ile Tyr Leu Ala Leu Gln Asp Ser Gly Leu Glu Val Asn Ile Val Thr Ala Ser Gln Tyr Ala Leu Gly Ile Ile Gln Ala Gln Pro Asp Gln Ser Glu Ser Glu Leu Val Asn Gln Ile Ile Glu Gln Leu Ile Lys Lys Glu Lys Val Tyr Leu Ala Trp Val Pro Ala His Lys Gly Ile Gly Gly Asn Glu Gln Val Asp Lys Leu Val Ser Ala Gly Ile Arg Lys Val Leu Phe Leu Asp Gly Ile Asp Lys Ala Gln Asp Glu His Glu Lys Tyr His Ser Asn Trp Arg Ala Met Ala Ser Asp Phe Asn Leu Pro Pro Val Val Ala Lys Glu Ile Val Ala Ser Cys Asp Lys Cys Gln Leu Lys Gly Glu Ala Met His Gly Gln Val Asp Cys Ser Pro Gly Ile Trp Gln Leu Ala Cys Thr His Leu Glu Gly Lys Val Ile Leu Val Ala Val His Val Ala Ser Gly Tyr Ile Glu Ala Glu Val Ile Pro Ala Glu Thr Gly Gln Glu Thr Ala Tyr Phe Leu Leu Lys Leu Ala Gly Arg Trp Pro Val Lys Thr Ile His Thr Ala Asn Gly Ser Asn Phe Thr Gly Ala Thr Val Arg Ala Ala Cys Trp Trp Ala Gly Ile Lys Gln Glu Phe Gly Ile Pro Tyr Asn Pro Gln Ser Gln Gly Val Val Ala Ser Met Asn Lys Glu Leu Lys Lys Ile Ile Gly Gln Val Arg Asp Gln Ala Glu His Leu Lys Thr Ala Val Gln Met Ala Val Phe Ile His Asn Phe Lys Arg Lys Gly Gly Ile Gly Gly Tyr Ser Ala Gly Glu Arg Ile Val Asp Ile Ile Ala Thr Asp Ile Gln Thr Lys Glu Leu Gln Lys Gln Ile Thr Lys Ile Gln Asn Phe Arg Val Tyr Tyr Arg Asp Ser Arg Asn Pro Leu Trp Lys Gly Pro Ala Lys Leu Leu Trp Lys Gly Glu Gly Ala Val Val Ile Gln Asp Asn Ser Asp Ile Lys Val Val Pro Arg Arg Lys Ala Lys Ile Ile Arg Asp Tyr Gly Lys Gln Met Ala Gly Asp Asp Cys Val Ala Ser Arg Gln Asp Glu Asp SEQ ID NO: 39

CORRECTED VERSION

(19) World Intellectual Property Organization International Bureau





(43) International Publication Date 21 March 2002 (21.03.2002)

PCT

(10) International Publication Number WO 02/022080 A3

(51) International Patent Classification7:

. . .

- (21) International Application Number: PCT/US01/28861
- (22) International Filing Date:

14 September 2001 (14.09.2001)

(25) Filing Language:

English

C12N 15/86

(26) Publication Language:

English

- (30) Priority Data: 60/233,180 15 September 2000 (15.09.2000) US
- (71) Applicant (for all designated States except US): MERCK & CO., INC. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US).
- (72) Inventors; and
- (75) Inventors/Applicants (for US only): EMINI, Emilio, A. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). YOUIL, Rima [AU/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). BETT, Andrew, J. [CA/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). CHEN, Ling [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). KASLOW, David, C. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). SHIVER, John, W. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). CASIMIRO, Daniel, R. [PH/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US).
- (74) Common Representative: MERCK & CO., INC.; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US).

- (81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.
- (84) Designated States (regional): ARIPO patent (GII, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

- with international search report
- (88) Date of publication of the international search report:

 2 May 2002

Date of publication of the revised international search report: 16 January 2003

(48) Date of publication of this corrected version:

6 March 2003

(15) Information about Corrections:

see PCT Gazette No. 10/2003 of 6 March 2003, Section II Previous Corrections:

see PCT Gazette No. 03/2003 of 16 January 2003, Section II

see PCT Gazette No. 30/2002 of 25 July 2002, Section II

[Continued on next page]

(54) Title: ENHANCED FIRST GENERATION ADENOVIRUS VACCINES EXPRESSING CODON OPTIMIZED HIV1-GAG, POL, NEF AND MODIFICATIONS

(57) Abstract: First generation adenoviral vectors and associated recombinant adenovirus-based HIV vaccines which show enhanced stability and growth properties and greater cellular-mediated immunity are described within this specification. These adenoviral vectors are utilized to generate and produce through cell culture various adenoviral-based HIV-1 vaccines which contain HIV-1 gag, HIV-1 pol and/or HIV-1 nef polynucleotide pharmaceutical products, and biologically relevant modifications thereof. These adenovirus vaccines, when directly introduced into living vertebrate tissue, preferably a mammalian host such as a human or a non-human mammal of commercial or domestic veterinary importance, express the HIV-1 Gag, Pol and/or Nef protein or biologically modification thereof, inducing a cellular immune response which specifically recognizes HIV-1. The exemplified polynucleotides of the present invention are synthetic DNA molecules encoding HIV-1 Gag, encoding codon optimized HIV-1 Pol, derivatives of optimized HIV-1 Pol (including constructs wherein protease, reverse transcriptase, RNAse H and integrase activity of HIV-1 Pol is inactivated), HIV-1 Nef and derivatives of optimized HIV-1 Nef, including nef mutants which effect wild type characteristics of Nef, such as myristylation and down regulation of host CD4. The adenoviral vaccines of the present invention, when administered alone or in a combined modality regime, will offer a prophylactic advantage to previously uninfected individuals and/or provide a therapeutic effect by reducing viral load levels within an infected individual, thus prolonging the asymptomatic phase of HIV-1 infection.



For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

TITLE OF THE INVENTION

ENHANCED FIRST GENERATION ADENOVIRUS VACCINES EXPRESSING CODON OPTIMIZED HIV1-GAG, POL, NEF AND MODIFICATIONS

5 CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit, under 35 U.S.C. §119(e), of U.S. provisional applications 60/233,180, 60/279,056, and Attorney Docket 20867PV2 (serial number unassigned), filed September 15, 2000, March 27, 2001, and September 7, 2001, respectively.

10

15

20

25

30

35

STATEMENT REGARDING FEDERALLY-SPONSORED R&D Not Applicable

REFERENCE TO MICROFICHE APPENDIX

Not Applicable

FIELD OF THE INVENTION

The present invention relates to recombinant, replication-deficient first generation adenovirus vaccines found to exhibit enhanced growth properties and greater cellular-mediated immunity as compared to other replication-deficient vectors. The invention also relates to the associated first generation adenoviral vectors described herein, which, through the incorporation of additional 5' adenovirus sequence, enhance large scale production efficiency of the recombinant, replicationdefective adenovirus described herein. Another aspect of the instant invention is the surprising discovery that the intron A portion of the human cytomegalovirus (hCMV) promoter constitutes a region of instability in adenoviral vector constructs. Removal of this region from adenoviral expression constructs results in greatly improved vector stability. Therefore, improved vectors expressing a transgene under the control of an intron A-deleted CMV promoter constitute a further aspect of this invention. These adenoviral vectors are useful for generating recombinant adenovirus vaccines against human immunodeficiency virus (HIV). In particular, the first generation adenovirus vectors disclosed herein are utilized to construct and generate adenovirus-based HIV-1 vaccines which contain HIV-1 Gag, HIV-1 Pol and/or HIV-1 Nef polynucleotide pharmaceutical products, and biologically active modifications thereof. Host administration of the recombinant, replication-deficient adenovirus vaccines described herein results in expression of HIV-1 Gag, HIV-1- Pol and/or Nef protein or

immunologically relevant modifications thereof, inducing a cellular immune response which specifically recognizes HIV-1. The exemplified polynucleotides of the present invention are synthetic DNA molecules encoding codon optimized HIV-1 Gag, HIV-1 Pol, derivatives of optimized HIV-1 Pol (including constructs wherein protease, reverse transcriptase, RNAse H and integrase activity of HIV-1 Pol is inactivated), HIV-1 Nef, and derivatives of optimized HIV-1 Nef, including nef mutants which effect wild type characteristics of Nef, such as myristylation and down regulation of host CD4. The HIV adenovirus vaccines of the present invention, when administered alone or in a combined modality and/or prime/boost regimen, will offer a prophylactic advantage to previously uninfected individuals and/or provide a therapeutic effect by reducing viral load levels within an infected individual, thus prolonging the asymptomatic phase of HIV-1 infection.

BACKGROUND OF THE INVENTION

10

15

20

25

30

Human Immunodeficiency Virus-1 (HIV-1) is the etiological agent of acquired human immune deficiency syndrome (AIDS) and related disorders. HIV-1 is an RNA virus of the Retroviridae family and exhibits the 5'LTR-gag-pol-env-LTR 3'organization of all retroviruses. The integrated form of HIV-1, known as the provirus, is approximately 9.8 Kb in length. Each end of the viral genome contains flanking sequences known as long terminal repeats (LTRs). The HIV genes encode at least nine proteins and are divided into three classes; the major structural proteins (Gag, Pol, and Env), the regulatory proteins (Tat and Rev); and the accessory proteins (Vpu, Vpr, Vif and Nef).

The gag gene encodes a 55-kilodalton (kDa) precursor protein (p55) which is expressed from the unspliced viral mRNA and is proteolytically processed by the HIV protease, a product of the pol gene. The mature p55 protein products are p17 (matrix), p24 (capsid), p9 (nucleocapsid) and p6.

The pol gene encodes proteins necessary for virus replication; a reverse transcriptase, a protease, integrase and RNAse H. These viral proteins are expressed as a Gag-Pol fusion protein, a 160 kDa precursor protein which is generated via a ribosomal frame shifting. The viral encoded protease proteolytically cleaves the Pol polypeptide away from the Gag-Pol fusion and further cleaves the Pol polypeptide to the mature proteins which provide protease (Pro, P10), reverse transcriptase (RT, P50), integrase (IN, p31) and RNAse H (RNAse, p15) activities.

The *nef* gene encodes an early accessory HIV protein (Nef) which has been shown to possess several activities such as down regulating CD4 expression, disturbing T-cell activation and stimulating HIV infectivity.

The env gene encodes the viral envelope glycoprotein that is translated as a 160-kilodalton (kDa) precursor (gp160) and then cleaved by a cellular protease to yield the external 120-kDa envelope glycoprotein (gp120) and the transmembrane 41-kDa envelope glycoprotein (gp41). Gp120 and gp41 remain associated and are displayed on the viral particles and the surface of HIV-infected cells.

5

10

15

20

25

30

35

The tat gene encodes a long form and a short form of the Tat protein, a RNA binding protein which is a transcriptional transactivator essential for HIV-1 replication.

The rev gene encodes the 13 kDa Rev protein, a RNA binding protein. The Rev protein binds to a region of the viral RNA termed the Rev response element (RRE). The Rev protein promotes transfer of unspliced viral RNA from the nucleus to the cytoplasm. The Rev protein is required for HIV late gene expression and in turn, HIV replication.

Gp120 binds to the CD4/chemokine receptor present on the surface of helper T-lymphocytes, macrophages and other target cells in addition to other co-receptor molecules. X4 (macrophage tropic) virus show tropism for CD4/CXCR4 complexes while a R5 (T-cell line tropic) virus interacts with a CD4/CCR5 receptor complex. After gp120 binds to CD4, gp41 mediates the fusion event responsible for virus entry. The virus fuses with and enters the target cell, followed by reverse transcription of its single stranded RNA genome into the double-stranded DNA via a RNA dependent DNA polymerase. The viral DNA, known as provirus, enters the cell nucleus, where the viral DNA directs the production of new viral RNA within the nucleus, expression of early and late HIV viral proteins, and subsequently the production and cellular release of new virus particles. Recent advances in the ability to detect viral load within the host shows that the primary infection results in an extremely high generation and tissue distribution of the virus, followed by a steady state level of virus (albeit through a continual viral production and turnover during this phase), leading ultimately to another burst of virus load which leads to the onset of clinical AIDS. Productively infected cells have a half life of several days, whereas chronically or latently infected cells have a 3-week half life, followed by non-productively infected cells which have a long half life (over 100 days) but do not significantly contribute to day to day viral loads seen throughout the course of disease.

Destruction of CD4 helper T lymphocytes, which are critical to immune defense, is a major cause of the progressive immune dysfunction that is the hallmark of HIV infection. The loss of CD4 T-cells seriously impairs the body's ability to fight most invaders, but it has a particularly severe impact on the defenses against viruses, fungi, parasites and certain bacteria, including mycobacteria.

5

10

15

20

25

30

35

Effective treatment regimens for HIV-1 infected individuals have become available recently. However, these drugs will not have a significant impact on the disease in many parts of the world and they will have a minimal impact in halting the spread of infection within the human population. As is true of many other infectious diseases, a significant epidemiologic impact on the spread of HIV-1 infection will only occur subsequent to the development and introduction of an effective vaccine. There are a number of factors that have contributed to the lack of successful vaccine development to date. As noted above, it is now apparent that in a chronically infected person there exists constant virus production in spite of the presence of anti-HIV-1 humoral and cellular immune responses and destruction of virally infected cells. As in the case of other infectious diseases, the outcome of disease is the result of a balance between the kinetics and the magnitude of the immune response and the pathogen replicative rate and accessibility to the immune response. Pre-existing immunity may be more successful with an acute infection than an evolving immune response can be with an established infection. A second factor is the considerable genetic variability of the virus. Although anti-HIV-1 antibodies exist that can neutralize HIV-1 infectivity in cell culture, these antibodies are generally virus isolate-specific in their activity. It has proven impossible to define serological groupings of HIV-1 using traditional methods. Rather, the virus seems to define a serological "continuum" so that individual neutralizing antibody responses, at best, are effective against only a handful of viral variants. Given this latter observation, it would be useful to identify immunogens and related delivery technologies that are likely to elicit anti-HIV-1 cellular immune responses. It is known that in order to generate CTL responses antigen must be synthesized within or introduced into cells, subsequently processed into small peptides by the proteasome complex, and translocated into the endoplasmic reticulum/Golgi complex secretory pathway for eventual association with major histocompatibility complex (MHC) class I proteins. CD8⁺ T lymphocytes recognize antigen in association with class I MHC via the T cell receptor (TCR) and the CD8 cell surface protein. Activation of naive CD8⁺ T cells into activated effector or memory cells generally requires both TCR engagement of antigen as described above as well as engagement of costimulatory proteins. Optimal

induction of CTL responses usually requires "help" in the form of cytokines from CD4⁺ T lymphocytes which recognize antigen associated with MHC class II molecules via TCR and CD4 engagement.

5

10

15

20

25

30

35

European Patent Applications 0 638 316 (Published February 15, 1995) and 0 586 076 (Published March 9, 1994), (both assigned to American Home Products Corporation) describe replicating adenovirus vectors carrying an HIV gene, including env or gag. Various treatment regimens were used with chimpanzees and dogs, some of which included booster adenovirus or protein plus alum treatments.

Replication-defective adenoviral vectors harboring deletions in the E1 region are known, and recent adenoviral vectors have incorporated the known packaging repeats into these vectors; e.g., see EP 0 707 071, disclosing, *inter alia*, an adenoviral vector deleted of E1 sequences from base pairs 459 to 3328; and U.S. Patent No. 6,033,908, disclosing, *inter alia*, an adenoviral vector deleted of base pairs 459-3510. The packaging efficiency of adenovirus has been taught to depend on the number of incorporated individual A (packaging) repeats; *see*, *e.g.*, Gräble and Hearing, 1990 J. Virol. 64(5):2047-2056; Gräble and Hearing, 1992 J. Virol. 66(2):723-731.

Larder, et al., (1987, Nature 327: 716-717) and Larder, et al., (1989, Proc. Natl. Acad. Sci. 86: 4803-4807) disclose site specific mutagenesis of HIV-1 RT and the effect such changes have on in vitro activity and infectivity related to interaction with known inhibitors of RT.

Davies, et al. (1991, Science 252:, 88-95) disclose the crystal structure of the RNase H domain of HIV-1 Pol.

Schatz, et al. (1989, FEBS Lett. 257: 311-314) disclose that mutations Glu478Gln and His539Phe in a complete HIV-1 RT/RNase H DNA fragment results in defective RNase activity without effecting RT activity.

Mizrahi, et al. (1990, *Nucl. Acids. Res.* 18: pp. 5359-5353) disclose additional mutations Asp443Asn and Asp498Asn in the RNase region of the *pol* gene which also results in defective RNase activity. The authors note that the Asp498Asn mutant was difficult to characterize due to instability of this mutant protein.

Leavitt, et al. (1993, J. Biol. Chem. 268: 2113-2119) disclose several mutations, including a Asp64Val mutation, which show differing effect on HIV-1 integrase (IN) activity.

Wiskerchen, et al. (1995, J. Virol. 69: 376-386) disclose singe and double mutants, including mutation of aspartic acid residues which effect HIV-1 IN and viral replication functions.

It would be of great import in the battle against AIDS to produce a prophylactic- and/or therapeutic-based HTV vaccine which generates a strong cellular immune response against an HIV infection. The present invention addresses and meets these needs by disclosing a class of adenovirus vaccines which, upon host administration, express codon optimized and modified versions of the HIV-1 genes, gag, pol and nef. These recombinant, replication-defective adenovirus vaccines may be administered to a host, such as a human, alone or as part of a combined modality regimen and/or prime-boost vaccination regimen with components of the present invention and/or a distinct viral HIV DNA vaccine, non-viral HIV DNA vaccine, HIV subunit vaccine, an HIV whole killed vaccine and/or a live attenuated HIV vaccine.

SUMMARY OF THE INVENTION

10

15

20

30

35

The present invention relates to enhanced replication-defective recombinant adenovirus vaccine vectors and associated recombinant, replication-deficient adenovirus vaccines which encode various forms of HIV-1 Gag, HIV-1 Pol, and/or HIV-1 Nef, including immunologically relevant modifications of HIV-1 Gag, HIV-1 Pol and HIV-1 Nef. The adenovirus vaccines of the present invention express HIV antigens and provide for improved cellular-mediated immune responses upon host administration. Potential vaccinees include but are not limited to primates and especially humans and non-human primates, and also include any non-human mammal of commercial or domestic veterinary importance. An effect of the improved recombinant adenovirus-based vaccines of the present invention should be a lower transmission rate to previously uninfected individuals (i.e., prophylactic applications) and/or reduction in the levels of the viral loads within an infected 25 individual (i.e., therapeutic applications), so as to prolong the asymptomatic phase of HIV-1 infection. In particular, the present invention relates to adenoviral-based vaccines which encode various forms of codon optimized HIV-1 Gag (including but in no way limited to p55 versions of codon optimized full length (FL) Gag and tPA-Gag fusion proteins), HIV-1 Pol, HIV-1 Nef, and selected modifications of immunological relevance. The administration, intracellular delivery and expression of these adenovirus vaccines elicit a host CTL and Th response. The preferred replication-defective recombinant adenoviral vaccine vectors include but are not limited to synthetic DNA molecules which (1) encode codon optimized versions of wild type HIV-1 Gag; (2) encode codon optimized versions of HIV-1 Pol; (3) encode codon optimized versions of HIV-1 Pol fusion proteins; (4) encode codon optimized versions of modified HIV-1 Pol proteins and fusion proteins, including but not limited

to pol modifications involving residues within the catalytic regions responsible for RT, RNase and IN activity within the host cell; (5) encode codon optimized versions of wild type HIV-1 Nef; (6) codon optimized versions of HIV-1 Nef fusion proteins; and/or (7) codon optimized versions of HIV-1 Nef derivatives, including but not limited to nef modifications involving introduction of an amino-terminal leader sequence, removal of an amino-terminal myristylation site and/or introduction of dileucine motif mutations. The Nef-based fusion and modified proteins, disclosed within this specification and expressed from an adenoviral-based vector vaccine this specification, may possess altered trafficking and/or host cell function while retaining the ability to be properly presented to the host MHC I complex and in turn elicit a host CTL and Th response. Examples of HIV-1 Gag, Pol and/or Nef fusion proteins include but are not limited to fusion of a leader or signal peptide at the NH₂-teriminal portion of the viral antigen coding region. Such a leader peptide includes but is not limited to a tPA leader peptide.

5

10

15

20

25

30

35

The adenoviral vector utilized in construction of the HIV-1 Gag-, HIV-1 Poland/or HIV-1 Nef- based vaccines of the present invention may comprise any replication-defective adenoviral vector which provides for enhanced genetic stability of the recombinant adenoviral genome through large scale production and purification of the recombinant virus. In other words, an HIV-1 Gag-, Pol- or Nef-based adenovirus vaccine of the present invention is a purified recombinant, replicationdefective adenovirus which is shown to be genetically stable through multiple passages in cell culture and remains so during large scale production and purification procedures. Such a recombinant adenovirus vector and harvested adenovirus vaccine lends itself to large scale dose filling and subsequent worldwide distribution procedures which will be demanded of an efficacious monovalent or multivalent HIV vaccine. The present invention meets this basic requirement with description of a replication-defective adenoviral vector and vectors derived therefrom, at least partially deleted in E1, comprising a wildtype adenovirus cis-acting packaging region from about base pair 1 to between from about base pair 342 (more preferably, 400) to about base pair 458 of the wildtype adenovirus genome. A preferred embodiment of the instant invention comprises base pairs 1-450 of a wildtype adenovirus. In other preferred embodiments, the replication -defective adenoviral vector has, in addition thereto, a region 3' to the E1-deleted region comprising base pairs 3511-3523. Basepairs 342-450 (more particularly, 400-450) constitute an extension of the 5'region of previously disclosed vectors carrying viral antigens, particularly HTV antigens (see, e.g., PCT International Application PCT/US00/18332, published

January 11, 2001 (WO 01/02067), which claims priority to U.S. Provisional Application Serial Nos. 60/142,631 and 60/148,981, filed 7/6/1999 and 8/13/1999, respectively; these documents herein incorporated by reference. Applicants have found that extending the 5' region further into the E1 gene into the disclosed vaccine vectors incorporated elements found to be important in optimizing the packaging of the virus.

As compared to previous vectors not comprising basepairs from about 1 to between from about base pair 342 (more preferably, 400) to about base pair 458 of the wildtype adenovirus genome, vectors comprising the above region exhibited enhanced growth characteristics, with approximately 5-10 fold greater amplification rates, a 10 more potent virus effect, allowing lower doses of virus to be used to generate equivalent immunity; and a greater cellular-mediated immune response than replication-deficient vectors not comprising this region (basepairs 1-450). Even more important, adenoviral constructs derived therefrom are very stable genetically in 15 large-scale production, particularly those comprising an expression cassette under the control of a hCMV promoter devoid of intron A. This is because Applicants have surprisingly found that the intron A portion of the hCMV promoter constituted a region of instability when employed in adenoviral vectors. Applicants have, therefore, identified an enhanced adenoviral vector which is particularly suited for use 20 in gene therapy and nucleotide-based vaccine vectors which, favorably, lends itself to large scale propagation.

A preferred embodiment of this invention is a replication-defective adenoviral vector in accordance with the above description wherein the gene is inserted in the form of a gene expression cassette comprising (a) a nucleic acid encoding a protein or biologically active and/or immunologically relevant portion thereof; (b) a heterologous promoter operatively linked to the nucleic acid of part a); and, (c) a transcription terminator.

25

30

35

In preferred embodiments, the E1 gene, other than that contained within basepairs 1-450 or, alternatively, that contained within base pairs 1-450 and 3511-3523 has been deleted from the adenoviral vector, and the gene expression cassette has replaced the deleted E1 gene. In other preferred embodiments, the replication defective adenovirus genome does not have a functional E3 gene, or the E3 gene has been deleted. Most preferably, the E3 region is present within the adenoviral genome. Further preferred embodiments are wherein the gene expression cassette is in an E1 anti-parallel (transcribed in a 3' to 5' direction relative to the vector backbone)

orientation or, more preferably, an E1 parallel (transcribed in a 5' to 3' direction relative to the vector backbone) orientation.

5

10

15

20

25

30

35

Further embodiments relate to a shuttle plasmid vector comprising: an adenoviral portion and a plasmid portion, wherein said adenovirus portion comprises: a) a replication defective adenovirus genome, at least partially deleted in E1, comprising a wildtype adenovirus cis-acting packaging region from about base pair 1 to between from about base pair 342 (more preferably, 400) to about base pair 458 (preferably, 1-450) of the wildtype adenovirus genome and, preferably, in addition thereto, basepairs 3511-3523 of a wildtype adenovirus sequence; and b) a gene expression cassette comprising: (a) a nucleic acid encoding a protein or biologically active and/or immunologically relevant portion thereof; (b) a heterologous promoter operatively linked to the nucleic acid of part a); and (c) a transcription terminator and/or a polyadenylation site.

Other aspects of this invention include a host cell comprising said adenoviral vectors and/or said shuttle plasmid vectors; vaccine compositions comprising said vectors; and methods of producing the vectors comprising (a) introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and (b) harvesting the resultant adenoviral vectors.

To this end, the present invention particularly relates to harvested recombinant, replication defective virus derived from a host cell, such as but not limited to 293 cells or PER.C6® cells, including but not limited to harvested virus related to any of the MRKAd5 vector backbones, with or without an accompanying transgene, including but not limited to the HIV-1 antigens described herein. An HIV-1 vaccine is represented by any harvested, recombinant adenovirus material which expresses any one or more of the HIV-1 antigens disclosed herein. This harvested material may then be purified, formulated and stored prior to host administration.

Another aspect of this invention is a method of generating a cellular immune response against a protein in an individual comprising administering to the individual an adenovirus vaccine vector comprising:

a) a recombinant, replication defective adenoviral vector, at least partially deleted in E1, comprising a wildtype adenovirus *cis*-acting adenovirus packaging region from about base pair 1 to between from about base pair 342 (more preferably, 400) to about base pair 458 (preferably, 1-450) and, preferably in addition thereto, base pairs 3511-3523 of a wildtype adenovirus sequence, and,

b) a gene expression cassette comprising:(i) a nucleic acid encoding a protein or biologically active and/or immunologically relevant portion thereof; (ii) a heterologous promoter operatively linked to the nucleic acid of part a); and (iii) a transcription terminator and/or a polyadenylation site.

5

10

15

20

25

30

35

In view of the efficacious nature of the adenoviral and/or DNA plasmid vaccines described herein, the present invention relates to all methodology regarding administration of one or more of these adenoviral and/or DNA plasmid vaccines to provide effective immunoprophylaxis, to prevent establishment of an HIV-1 infection following exposure to this virus, or as a post-HIV infection therapeutic vaccine to mitigate the acute HIV-1 infection so as to result in the establishment of a lower virus load with beneficial long term consequences. As discussed herein, such a treatment regimen may include a monovalent or multivalent composition, various combined modality applications, and/or a prime/boost regimen to as to optimize antigen expression and a concomitant cellular-mediated and/or humoral immune response upon inoculation into a living vertebrate tissue. Therefore, the present invention provides for methods of using the adenoviral and/or DNA plasmid vaccines disclosed herein within the various parameters disclosed herein as well as any additional parameters known in the art, which, upon introduction into mammalian tissue induces intracellular expression of the gag, pol and/or nef-based vaccines.

To this end, the present invention relates in part to methods of generating a cellular immune response in a vaccinee, preferably a human vaccinee, wherein the individual is given more than one administration of adenovirus vaccine vector, and it may be given in a regimen accompanied by the administration of a plasmid vaccine. The plasmid vaccine (also referred to herein as a "DNA plasmid vaccine" or "vaccine plasmid" comprises a nucleic acid encoding a protein or an immunologically relevant portion thereof, a heterologous promoter operably linked to the nucleic acid sequence, and a transcription terminator or a polyadenylation signal (such as bGH or SPA, respectively). There may be a predetermined minimum amount of time separating the administrations. The individual can be given a first dose of plasmid vaccine, and then a second dose of plasmid vaccine. Alternatively, the individual may be given a first dose of adenovirus vaccine, and then a second dose of adenovirus vaccine. In other embodiments, the plasmid vaccine is administered first, followed after a time by administration of the adenovirus vaccine. Conversely, the adenovirus vaccine may be administered first, followed by administration of plasmid vaccine after a time. In these embodiments, an individual may be given multiple doses of the same adenovirus serotype in either viral vector or plasmid form, or the virus may be of

differing serotypes. In the alternative, a viral antigen of interest can be first delivered via a viral vaccine other than an adenovirus-based vaccine, and then followed with the adenoviral vaccine disclosed. Alternative viral vaccines include but are not limited to pox virus and venezuelan equine encephilitis virus.

5

10

15

20

25

30

35

The present invention also relates to multivalent adenovirus vaccine compositions which comprise Gag, Pol and Nef components described herein; see, e.g., Example 29 and Table 25. Such compositions will provide for an enhanced cellular immune response subsequent to host administration, particularly given the genetic diversity of human MHCs and of circulating virus. Examples, but not limitations, include MRKAd5-vector based multivalent vaccine compositions which provide for a divalent (i.e., gag and nef, gag and pol, or pol and nef components) or a trivalent vaccine (i.e., gag, pol and nef components) composition. Such a mutlivalent vaccine may be filled for a single dose or may consist of multiple inoculations of each individually filled component; and may in addition be part of a prime/boost regimen with viral or non-viral vector vaccines as introduced in the previous paragraph. To this end, preferred compositions are MRKAd5 adenovirus used in combination with multiple, distinct HIV antigen classes. Each HIV antigen class is subject to sequence manipulation, thus providing for a multitude of potential vaccine combinations; and such combinations are within the scope of the present invention. The utilization of such combined modalities vaccine formulation and administration increase the probability of eliciting an even more potent cellular immune response when compared to inoculation with a single modality regimen.

The concept of a "combined modality" as disclosed herein also covers the alternative mode of administration whereby multiple HIV-1 viral antigens may be ligated into a proper shuttle plasmid for generation of a pre-adenoviral plasmid comprising multiple open reading frames. For example, a trivalent vector may comprise a gag-pol-nef fusion, in either a E3(-) or E3(+) background, preferably a E3 deleted backbone, or possibly a "2+1" divalent vaccine, such as a gag-pol fusion (i.e., codon optimized p55 gag and inactivated optimized pol; Example 29 and Table 25) within the same MRKAd5 backbone, with each open reading frame being operatively linked to a distinct promoter and transcription termination sequence. Alternatively, the two open reading frames may be operatively linked to a single promoter, with the open reading frames operatively linked by an internal ribosome entry sequence (IRES). Therefore, a multivalent vaccine delivered as a single, or possibly a second harvested recombinant, replication-deficient adenovirus is contemplated as part of the present invention.

Therefore, the adenoviral vaccines and plasmid DNA vaccines of this invention may be administered alone, or may be part of a prime and boost administration regimen. A mixed modality priming and booster inoculation scheme will result in an enhanced immune response, particularly if pre-existing anti-vector immune responses are present. This one aspect of this invention is a method of priming a subject with the plasmid vaccine by administering the plasmid vaccine at least one time, allowing a predetermined length of time to pass, and then boosting by administering the adenoviral vaccine. Multiple primings typically, 1-4, are usually employed, although more may be used. The length of time between priming and boost may typically vary from about four months to a year, but other time frames may be used. In experiments with rhesus monkeys, the animals were primed four times with plasmid vaccines, then were boosted 4 months later with the adenoviral vaccine. Their cellular immune response was notably higher than that of animals which had only received adenoviral vaccine. The use of a priming regimen may be particularly preferred in situations where a person has a pre-existing anti-adenovirus immune response.

5

10

15

20

30

35

It is an object of the present invention to provide for enhanced replication-defective recombinant adenoviral vaccine vector backbones. These recombinant adenoviral backbones may accept one or more transgenes, which may be passaged through cell culture for growth, amplification and harvest.

It is a further object to provide for enhanced replication-defective recombinant adenoviral vaccine vectors which encode various transgenes.

It is also an object of the present invention to provide for a harvested recombinant, replication-deficient adenovirus which shows enhanced growth and amplification rates while in combination with increased virus stability after continuous passage in cell culture. Such a recombinant adenovirus is particularly suited for use in gene therapy and nucleotide-based vaccine vectors which, favorably, lends itself to large scale propagation.

To this end, it is an object of the present invention to provide for (1) enhanced replication-defective recombinant adenoviral vaccine vectors as described herein which encode various forms of HIV-1 Gag, HIV-1 Pol, and/or HIV-1 Nef, including immunologically relevant modifications of HIV-1 Gag, HIV-1 Pol and HIV-1 Nef, and (2) harvested, purified recombinant replication-deficient adenovirus generated by passage of the adenoviral vectors of (1) through one or multiple passages through cell culture, including but not limited to passage through 293 cells or PER.C6® cells.

It is also an object of the present invention to provide for recombinant adenovirus harvested by one or multiple passages through cell culture. As relating to recombinant adenoviral vaccine vector, this recombinant virus is harvested and formulated for subsequent host administration.

It is also an object of the present invention to provide for replication-defective adenoviral vectors wherein at least one gene is inserted in the form of a gene expression cassette comprising (a) a nucleic acid encoding a protein or biologically active and/or immunologically relevant portion thereof; (b) a heterologous promoter operatively linked to the nucleic acid of part a); and, (c) a transcription terminator.

5

10

15

20

25

30

35

It is also an object of the present invention to provide for a host cell comprising said adenoviral vectors and/or said shuttle plasmid vectors; vaccine compositions comprising said vectors; and methods of producing the vectors comprising (a) introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and (b) harvesting the resultant adenoviral vectors.

It is a further object of the present invention to provide for methods of generating a cellular immune response against a protein in an individual comprising administering to the individual an adenovirus vaccine vector comprising a) a replication defective

adenoviral vector, at least partially deleted in E1, comprising a wildtype adenovirus cis-acting packaging region from about base pair 1 to between from about base pair 342 (more preferably, 400) to about 450 (preferably, 1-450) and, preferably, 3511-3523 of a wildtype adenovirus sequence, and, b) a gene expression cassette comprising: (i) a nucleic acid encoding a protein or biologically active and/or immunologically relevant portion thereof; (ii) a heterologous promoter operatively linked to the nucleic acid of part a); and (iii) a transcription terminator and/or a polyadenylation site.

It is also an object of the present invention to provide various alternatives for vaccine administration regimes, namely administration of one or more adenoviral and/or DNA plasmid vaccines described herein to provide effective immunoprophylaxis for uninfected individuals or a therapeutic treatment for HIV infected patients. Such processes include but are not limited to multivalent HIV-1 vaccine compositions, various combined modality regimes as well as various prime/boost alternatives. These methods of administration, relating to vaccine composition and/or scheduled administration, will increase the probability of eliciting an even more potent cellular immune response when compared to inoculation with a single modality regimen.

As used throughout the specification and claims, the following definitions and abbreviations are used:

"HAART" refers to - highly active antiretroviral therapy -.

"first generation" vectors are characterized as being replication-defective.

They typically have a deleted or inactivated E1 gene region, and preferably have a deleted or inactivated E3 gene region as well.

"AEX" refers to Anion Exchange chromatography.

"QPA" refers to Quick PCR-based Potency Assay.

"bps" refers to basepairs.

10

15

20

25

30

35

"s" or "str" denotes that the transgene is in the E1 parallel or "straight" orientation.

"PBMCs" refers to peripheral blood monocyte cells.

"FL" refers to full length.

"FLgag" refers to a full-length optimized gag gene, as shown in Figure 2.

"Ad5-Flgag" refers to an adenovirus serotype 5 replication deficient virus which carries an expression cassette which comprises a full length optimized gag gene under the control of a CMV promoter.

"Promoter" means a recognition site on a DNA strand to which an RNA polymerase binds. The promoter forms an initiation complex with RNA polymerase to initiate and drive transcriptional activity. The complex can be modified by activating sequences such as enhancers or inhibiting sequences such as silencers.

"Leader" means a DNA sequence at the 5' end of a structural gene which is transcribed along with the gene. This usually results a protein having an N-terminal peptide extension, often referred to as a pro-sequences.

"Intron" means a section of DNA occurring in the middle of a gene which does not code for an amino acid in the gene product. The precursor RNA of the intron is excised and is therefore not transcribed into mRNA not translated into protein.

"Immunologically relevant" or "biologically active" means (1) with regards to a viral protein, that the protein is capable, upon administration, of eliciting a measurable immune response within an individual sufficient to retard the propagation and/or spread of the virus and/or to reduce the viral load present within the individual; or (2) with regards to a nucleotide sequence, that the sequence is capable of encoding for a protein capable of the above.

"Cassette" refers to a nucleic acid sequence which is to be expressed, along with its transcription and translational control sequences. By changing the cassette, a vector can express a different sequence.

"bGHpA" refers to the bovine growth hormone transcription terminator/polyadenylation sequence.

"tPAgag" refers to a fusion between the leader sequence of the tissue plasminogen activator leader sequence and an optimized HIV gag gene, as exemplified in Figure 30A-B, whether in a DNA or adenovirus-based vaccine vector.

Where utilized, "IA" or "inact" refers to an <u>inactivated</u> version of a gene (e.g. IApol).

"MCS" is "multiple cloning site".

5

10

15

20

25

30

35

In general, adenoviral constructs, gene constructs are named by reference to the genes contained therein. For example:

"Ad5 HIV-1 gag", also referred to as the original HIV-1 gag adenoviral vector, is a vector containing a transgene cassette composed of a hCMV intron A promoter, the full length version of the human codon-optimized HIV-1 gag gene, and the bovine growth hormone polyadenylation signal. The transgene was inserted in the E1 antiparallel orientation in an E1 and E3 deleted adenovector.

"MRK Ad5 HIV-1 gag" also referred to as "MRKAd5gag" or "Ad5gag2" is an adenoviral vector taught herein which is deleted of E1, comprises basepairs 1-450 and 3511-3523, and has a human codon-optimized HIV-1 gene in an E1 parallel orientation under the control of a CMV promoter without intron A. The construct also comprises a bovine growth hormone polyadenylation signal.

"pV1JnsHIVgag", also referred to as "HIVFLgagPR9901", is a plasmid comprising the CMV immediate-early (IE) promoter and intron A, a full-length codon-optimized HIV gag gene, a bovine growth hormone-derived polyadenylation and transcriptional termination sequence, and a minimal pUC backbone.

"pV1JnsCMV(no intron)-FLgag-bGHpA" is a plasmid derived from pV1JnsHIVgag which is deleted of the intron A portion of CMV and which comprises the full length HIV gag gene. This plasmid is also referred to as "pV1JnsHIVgag-bGHpA", pV1Jns-hCMV-FL-gag-bGHpA" and "pV1JnsCMV(no intron) + FLgag + bGHpA".

"pV1JnsCMV(no intron)-FLgag-SPA" is a plasmid of the same composition as pV1JnsCMV(no intron)-FLgag-bGHpA except that the SPA termination sequence replaces that of bGHpA. This plasmid is also referred to as "pV1Jns-HIVgag-SPA" and pV1Jns-hCMV-FLgag-SPA".

"pdelE1sp1A" is a universal shuttle vector with no expression cassette (i.e., no promoter or polyA). The vector comprises wildtype adenovirus serotype 5 (Ad5) sequences from bp 1 to bp 341 and bp 3524 to bp 5798, and has a multiple cloning

site between the Ad5 sequences ending 341 bp and beginning 3524 bp. This plasmid is also referred to as the original Ad 5 shuttle vector.

"MRKpdelE1sp1A" or "MRKpdelE1(Pac/pIX/pack450)" or

5

10

15

20

25

30

35

"MRKpdelE1(Pac/pIX/pack450)Cla1" is a universal shuttle vector with no expression cassette (i.e. no promoter or polyA) comprising wildtype adenovirus serotype 5 (Ad5) sequences from bp1 to bp450 and bp 3511 to bp 5798. The vector has a multiple cloning site between the Ad5 sequence ending 450 bp and beginning 3511 bp. This shuttle vector may be used to insert the CMV promoter and the bGHpA fragments in both the straight ("str". or E1 parallel) orientation or in the opposite (opp. or E1 antiparallel) orientation)

"MRKpdelE1(Pac/pIX/pack450)+CMVmin+BGHpA(str.)" is still another shuttle vector which is the modified vector that contains the CMV promoter (no intronA) and the bGHpA fragments. The expression unit containing the hCMV promoter (no intron A) and the bovine growth hormone polyadenylation signal has been inserted into the shuttle vector such that insertion of the gene of choice at a unique BgIII site will ensure the direction of transcription of the transgene will be Ad5 E1 parallel when inserted into the MRKpAd5(E1/E3+)Cla1 pre-plasmid. This shuttle vector, as shown in Figures 22 and 23, was used to insert the respective IApol and G2A,LLAA nef genes directly into.

"MRKpdelE1-CMV(no intron)-FLgag-bGHpA" is a shuttle comprising Ad5 sequences from basepairs 1-450 and 3511-5798, with an expression cassette containing human CMV without intron A, the full-length human codon-optimized HIV gag gene and bovine growth hormone polyadenylation signal. This plasmid is also referred to as "MRKpdelE1 shuttle +hCMV-FL-gag-BGHpA"

"MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA" is an adenoviral vector comprising all Ad5 sequences except those nucleotides encompassing the E1 region (from 451-3510), a human CMV promoter without intron A, a full-length human codon-optimized HIV gag gene, and a bovine growth hormone polyadenylation signal. This vector is also referred to as "MRKpAdHVE3 + hCMV-FL-gag-BGHpA", "MRKpAd5HIV-1gag", "MRKpAd5gag", "pMRKAd5gag" or "pAd5gag2".

"pV1Jns-HIV-pol inact(opt)" or "pV1Jns-HIV IA pol (opt) is the inactivated Pol gene (contained within SEQ ID NO:3) cloned into the BgIII site of V1Jns (Figure 17A-C). As noted herein, various derivatives of HIV-1 pol may be cloned into a plasmid expression vector such as V1Jns or V1Jns-tPA, thus serving directly as DNA vaccine candidates or as a source for subcloning into an appropriate adenoviral vector.

"MRKpdel+hCMVmin+FL-pol+bGHpA(s)" is the "MRKpdelE1(Pac/pIX/pack450)+CMVmin+BGHpA(str.)" shuttle mentioned above which contains the IA pol gene is the proper orientation. This shuttle vector is used in a bacterial recombination with MRKpAd(E1-/E3+)Cla1.

"MRKpAd+hCMVmin+FL-pol+bGHpA(S)E3+", also referred to herein as "pMRKAd5pol", is the pre-adenovirus plasmid which comprises a CMV-pol inact(opt)-pGHpA construct. The construction of this pre-adenovirus plasmid is shown in Figure 22.

"pV1Jns/nef (G2A,LLAA)" or "V1Jns/opt nef (G2A,LLAA)" comprises codon optimized HIV-1 Nef wherein the open reading frame codes for modifications at the amino terminal myristylation site (Gly-2 to Ala-2) and substitution of the Leu-174-Leu-175 dileucine motif to Ala-174-Ala-175 (SEQ ID NO:13; which comprises an initiating methionine residue at nucleotides 12-14 and a "TAA" stop codon from nucleotides 660-662). This fragment is subcloned into the Bgl II site of V1Jns and/orV1Jns-tPA (Figures 16A-B). As noted above for HIV-1 pol, HIV-1 nef constructs may be cloned into a plasmid expression vector such as V1Jns or V1Jns-tPA, thus serving directly as DNA vaccine candidates or as a source for subcloning into an appropriate adenoviral vector.

"MRKpdelE1hCMVminFL-nefBGHpA(s)", also referred to herein as "pMRKAd5nef", is the pre-adenovirus plasmid which comprises a CMV-nef (G2A,LLAA) codon optimized sequence. The construction of this pre-adenovirus plasmid is shown in Figure 23.

BRIEF DESCRIPTION OF THE FIGURES

5

10

15

20

25

30

35

Figure 1 shows the original HIV-1 gag adenovector (Ad5HIV-1gag). This vector is disclosed in PCT International Application No. PCT/US00/18332 (WO 01/02607) filed July 3, 2000, claiming priority to U.S. Provisional Application Serial No. 60/142,631, filed July 6, 1999 and U.S. Application Serial No. 60/148,981, filed August 13, 1999, all three applications which are hereby incorporated by reference.

Figure 2 shows the nucleic acid sequence (SEQ ID NO: 29) of the optimized human HIV-1 gag open reading frame.

Figure 3 shows diagrammatically the new transgene constructs in comparison with the original gag transgene.

Figure 4 shows the modifications made to the original adenovector backbone in the generation of the novel vectors of the instant invention.

Figure 5 shows the virus mixing experiments that were carried out to determine the effects of the addition made to the packaging signal region (Expt. #1) and the E3 gene on viral growth (Expt. #2). The bars denote the region of modifications made to the E1 deletion.

Figure 6 shows an autoradiograph of viral DNA analysis following the viral mixing experiments described in Examples 6 and 7.

5

10

15

20

25

30

35

Figures 7A, 7B and 7C are as follows: Figure 7A shows the hCMV-Flgag-bGHpA adenovectors constructed within the MRKpAdHVE3 and MRKpAdHVO adenovector backbones. Both E1 parallel and E1 antiparallel transgene orientation are represented. Figure 7B shows the hCMV-Flgag-SPA adenovectors constructed within the MRKpAdHVE3 and MRKpAdHVO adenovector backbones. Again, both E1 parallel and E1 antiparallel transgene orientation are represented. Figure 7C shows the mCMV-Flgag-bGHpA adenovectors constructed within the MRKpAdHVE3 and MRKpAdHVO adenovector backbones. Once again, both E1 parallel and E1 antiparallel transgene orientation are represented.

Figure 8A shows the experiment designed to test the effect of transgene orientation.

Figure 8B shows the experiments designed to test the effect of polyadenylation signal.

Figure 9 shows viral DNA from the four adenoviral vectors tested (Example 12) at P5, following *Bst*E11 digestion.

Figure 10 shows viral DNA analysis of passages 11 and 12 of MRKpAdHVE3, MRKAd5HIV-1gag, and MRKAd5HIV-1gagE3-.

Figure 11 shows viral DNA analysis (*Hind*III digestion) of passage 6 MRKpAdHVE3 and MRKAd5HIV-1gag used to initiate the viral competition study. The last two lanes are passage 11 analysis of duplicate passages of the competition study (each virus at MOI of 280 viral particles).

Figure 12 shows viral DNA analysis by *Hind* III digestion on high passage numbers for MRKAd5HIV-1gag in serum-containing media with collections made at specified times. The first lane shows the 1kb DNA size marker. The other lanes represent pre-plasmid control (digested with Pac1 and *Hind*III), MRKAd5HIV-1gag at P16, P19, and P21.

Figure 13 shows serum anti-p24 levels at 3 wks post i.m. immunization of balb/c mice (n=10) with varying doses of several Adgag constructs: (A) MRK Ad5 HIV-1 gag (through passage 5); (B) MRKAd5 hCMV-FLgag-bGHpA (E3-); (C) MRKAd5 hCMV-FLgag-SPA (E3+); (D) MRKAd5 mCMV-FLgag-bGHpA (E3+);

(E) research lot (293 cell-derived) of Ad5HIV-1 gag; and (F) clinical lot (Ad5gagFN0001) of Ad5HIV-1 gag. Reported are the geometric mean titers (GMT) for each cohort along with the standard error bars.

Figure 14 shows a restriction map of the pMRKAd5HIV-1gag vector.

5

10

15

20

25

30

35

Figures 15A-X illustrates the nucleotide sequence of the pMRKAd5HIV-1gag vector (SEQ ID NO:27.[coding] and SEQ ID NO:28 [non-coding]).

Figures 16A-B shows a schematic representation of DNA vaccine expression vectors V1Jns (A) and V1Jns-tPA (B), which are utilized for HIV-1 gag, pol and nef constructs in various DNA/viral vector combined modality regimens as disclosed herein.

Figures 17A-C shows the nucleotide (SEQ ID NO:3) and amino acid sequence (SEQ ID NO:4) of IA-Pol. Underlined codons and amino acids denote mutations, as listed in Table 1.

Figure 18 shows codon optimized nucleotide and amino acid sequences through the fusion junction of tPA-pol inact(opt) (contained within SEQ ID NOs: 7 and 8, respectively). The underlined portion represents the NH₂-terminal region of IA-Pol.

Figures 19A-B show a nucleotide sequence comparison between wild type nef(jrfl) and codon optimized nef. The wild type nef gene from the jrfl isolate consists of 648 nucleotides capable of encoding a 216 amino acid polypeptide. WT, wild type sequence (SEQ ID NO:19); opt, codon-optimized sequence (contained within SEQ ID NO:1). The Nef amino acid sequence is shown in one-letter code (SEQ ID NO:2).

Figures 20A-C show nucleotide sequences at junctions between nef coding sequence and plasmid backbone of nef expression vectors V1Jns/nef (Figure 20A), V1Jns/nef(G2A,LLAA) (Figure 20B), V1Jns/tpanef (Figure 20C) and V1Jns/tpanef(LLAA) (Figure 20C, also). 5' and 3' flanking sequences of codon optimized nef or codon optimized nef mutant genes are indicated by bold/italic letters; nef and nef mutant coding sequences are indicated by plain letters. Also indicated (as underlined) are the restriction endonuclease sites involved in construction of respective nef expression vectors. V1Jns/tpanef and V1Jns/tpanef(LLAA) have identical sequences at the junctions.

Figure 21 shows a schematic presentation of nef and nef derivatives. Amino acid residues involved in Nef derivatives are presented. Glycine 2 and Leucine 174 and 175 are the sites involved in myristylation and dileucine motif, respectively. For both versions of the tpanef fusion genes, the putative leader peptide cleavage sites are

indicated with "*", and a exogenous serine residue introduced during the construction of the mutants is underlined.

Figure 22 shows diagrammatically the construction of the pre-adenovirus plasmid construct, MRKAd5Pol.

Figure 23 shows diagrammatically the construction of the pre-adenovirus plasmid construct, MRKAd5Nef.

5

10

15

20

25

30

35

Figure 24 shows a comparison of clade B vs. clade C anti-gag T cell responses in clade B HIV-infected subjects.

Figure 25 shows a comparison of clade B vs. clade C anti-nef T cell responses in clade B HIV-infected subjects.

Figures 26A-AO illustrates the nucleotide sequence of the pMRKAd5HIV-1pol adenoviral vector (SEQ ID NO:32 [coding] and SEQ ID NO:33 [non-coding]), comprising the coding region of the inactivated pol gene (SEQ ID NO3).

Figures 27A-AM illustrates the nucleotide sequence of the pMRKAd5HIV-1 nef adenoviral vector (SEQ ID NO:34 [coding] and SEQ ID NO:35 [non-coding]), comprising the coding region of the inactivated pol gene (SEQ ID NO13).

Figure 28 shows the stability of MRKAd5 vectors comprising various promoter fragments (hCMV or mCMV) and terminations signals (bGH or SPA) in E3(+) or E3(-) backbones.

Figures 29A and B shows the anion-exchange HPLC viral particle concentrations of the freeze-thaw recovered cell associated virus at the 24, 36, 48, and 60 hpi time points (Figure 29A) and the timcourse QPA supernatant titers (Figure 29B) for MRKAd5gag, MRKAd5pol and MRKAd5nef.

Figure 30 shows the nucleotide sequence (SEQ ID NO:36) and amino acid sequence (SEQ ID NO:37) comprising the open reading frame of a representative tPA-gag fusion for use in the DNA and/or adenoviral vaccine disclosed herein.

Figure 31 shows the intracellular γIFN staining of PBMCs collected at week 10 (post DNA prime) and week 30 (post Ad boost). The cells were stimulated overnight in the presence or absence of the gag peptide pool. They were subsequently stained using fluorescence-tagged anti-CD3, anti-CD8, anti-CD4, and anti-γIFN monoclonal antibodies. Each plot shows all CD3+ T cells which were segregated in terms of positive staining for surface CD8 and γIFN production. The numbers in the upper right and lower right quadrants of each plot are the percentages of CD3+ cells that were CD8+γIFN+ and CD4+γIFN+, respectively.

Figure 32 shows a comparison of single-modality adenovirus immunization with DNA + adjuvant prime/adenovirus boost immunization.

Figures 33A-B show the nucleotide sequence (SEQ ID NO: 38) of the open reading frame for the gag-IApol fusion of Example 29.

Figures 34A-B show the protein sequence (SEQ ID NO:39) of the gag-IApol fustion frame.

5

10

15

20

25

30

35

DETAILED DESCRIPTION OF THE INVENTION

A novel replication-defective, or "first generation," adenoviral vector suitable for use in gene therapy or nucleotide-based vaccine vectors is described. This vector is at least partially deleted in E1 and comprises a wildtype adenovirus cis-acting packaging region from about base pair 1 to between about base pair 342 (more preferably, 400) to about 458 (preferably, 1-450) and, preferably, 3511-3523 of a wild-type adenovirus sequence. It has been found that a vector of this description possesses enhanced growth characteristics, with approximately 5-10 fold greater amplification rates, and is more potent allowing lower doses of virus to be used to generate equivalent immunity. The vector, furthermore, generates a harvested recombinant adenovirus which shows greater cellular-mediated immune responses than replication-deficient vectors not comprising this region (basepairs 342-450). Adenoviral constructs derived from these vectors are, further, very stable genetically, particularly those comprising a transgene under the control of a hCMV promoter devoid of intron A. Viruses in accordance with this description were passaged continually and analyzed; see Example 12. Each virus analyzed maintained it correct genetic structure. Analysis was also carried out under propagation conditions similar to that performed in large scale production. Again, the vectors were found to possess enhanced genetic stability; see Figure 12. Following 21 passages, the viral DNA showed no evidence of rearrangement, and was highly reproducible from one production lot to the next. The outcome of all relevant tests indicate that the adenoviral vector is extremely well suited for large-scale production of recombinant, replication-deficient adenovirus, as shown herein with the data associated with Figure 28.

A preferred adenoviral vector in accordance with this description is a vector comprising basepairs 1-450, which is deleted in E3. This vector can accommodate up to approximately 7,500 base pairs of foreign DNA inserts (or exogenous genetic material). Another preferred vector is one retaining E3 which comprises basepairs 1-450. A preferred vector of this description is an E3+ vector comprising basepairs 1-450 and 3511-3523. This vector, when deleted of the region spanning basepairs 451-3510, can accommodate up to approximately, 4,850 base pairs of foreign DNA inserts

(or exogenous genetic material). The cloning capacities of the above vectors have been determined using 105% of the wildtype Ad5 sequence as the upper genome size limit.

Wildtype adenovirus serotype 5 is used as the basis for the specific basepair numbers provided throughout the specification. The wildtype adenovirus serotype 5 sequence is known and described in the art; see, Chroboczek et al., 1992 J. Virology 186:280, which is hereby incorporated by reference. Accordingly, a particular embodiment of the instant invention is a vector based on the adenovirus serotype 5 sequence. One of skill in the art can readily identify the above regions in other adenovirus serotypes (e.g., serotypes 2, 4, 6, 12, 16, 17, 24, 31, 33, and 42), regions defined by basepairs corresponding to the above basepair positions given for adenovirus serotype 5. Accordingly, the instant invention encompasses all adenoviral vectors partially deleted in E1 comprising basepairs corresponding to 1-450 (particularly, 342-450) and, preferably, 3511-3523 of a wild-type adenovirus serotype 5 (Ad5) nucleic acid sequence. Particularly preferred embodiments of the instant invention are those derived from adenoviruses like Ad5 which are classified in subgroup C (e.g., Ad2).

Vectors in accordance with the instant invention are at least partially deleted in E1. Preferably the E1 region is completely deleted or inactivated. Most preferably, the region deleted of E1 is within basepairs 451-3510. It is to be noted that the extended 5' and 3' regions of the disclosed vectors are believed to effectively reduce the size of the E1 deletion of previous constructs without overlapping any part of the E1A/E1B gene present in the cell line used, i.e., the PER.C6® cell line transefected with base pairs 459-3510. Overlap of adenoviral sequences is avoided because of the possibility of recombination. One of ordinary skill in the art can certainly appreciate that the instant invention can, therefore, be modified if a different cell line transfected with a different segment of adenovirus DNA is utilized. For purposes of exemplification, a 5' region of base pairs 1 to up to 449 is more appropriate if a cell line is transfected with adenoviral sequence from base pairs 450-3510. This holds true as well in the consideration of segments 3' to the E1 deletion.

Preferred embodiments of the instant invention possess an intact E3 region (i.e., an E3 gene capable of encoding a functional E3). Alternate embodiments have a partially deleted E3, an inactivated E3 region, or a sequence completely deleted of E3. Applicants have found, in accordance with the instant invention, that virus comprising the E3 gene were able to amplify more rapidly compared with virus not comprising an E3 gene; see Figure 6 wherein a diagnostic CsCl band corresponding to the E3+ virus

tested (5,665 bp) was present in greater amount compared with the diagnostic band of 3,010 bp corresponding to the E3- virus. These results were obtained following a virus competition study involving mixing equal MOI ratio (1:1) of adenovectors both comprising the E3 gene and not comprising the E3 gene. This increased amplification capacity of the E3+ adenovectors was subsequently confirmed with growth studies; see Table 4A, wherein the E3+ virus exhibit amplification ratios of 470, 420 and 320 as compared with the 115 and 40-50 of the E3- constructs.

5

10

15

20

25

30

35

As stated above, vectors in accordance with the instant invention can accommodate up to approximately 4,850 base pairs of exogenous genetic material for an E3+ vector and approximately 7,500 base pairs for an E3- vector. Preferably, the insert brings the adenoviral vector as close as possible to a wild-type genomic size (e.g., for Ad5, 35,935 basepairs). It is well known that adenovirus amplifies best when they are close to their wild-type genomic size.

The genetic material can be inserted in an E1-parallel or an E1 anti-parallel orientation, as such is illustrated in Figure 7A, 7B, 7C and Figure 8A. Particularly preferred embodiments of the instant invention, have the insert in an E1-parallel orientation. Applicants have found, via competition experiments with plasmids containing transgenes in differing orientation (Figure 8A), that vector constructs with the foreign DNA insert in an E1-parallel orientation amplify better and actually outcompete E1-antiparallel-oriented transgenes. Viral DNA analysis of the mixtures at passage 3 and certainly at passage 6, showed a greater ratio of the virus carrying the transgene in the E1 parallel orientation as compared with the E1 anti-parallel version. By passage 10, the only viral species observed was the adenovector with the transgene in the E1 parallel orientation for both transgenes tested.

Adenoviral vectors in accordance with the instant invention are particularly well suited to effectuate expression of desired proteins, one example of which is an HIV protein, particularly an HIV full length gag protein. Exogenous genetic material encoding a protein of interest can exist in the form of an expression cassette. A gene expression cassette preferably comprises (a) a nucleic acid encoding a protein of interest; (b) a heterologous promoter operatively linked to the nucleic acid encoding the protein; and (c) a transcription terminator.

The transcriptional promoter is preferably recognized by an eukaryotic RNA polymerase. In a preferred embodiment, the promoter is a "strong" or "efficient" promoter. An example of a strong promoter is the immediate early human cytomegalovirus promoter (Chapman et al, 1991 *Nucl. Acids Res*19:3979-3986, which is incorporated by reference), preferably without intronic sequences. Most preferred

for use within the instant adenoviral vector is a human CMV promoter without intronic sequences, like intron A. Applicants have found that intron A, a portion of the human cytomegalovirus promoter (hCMV), constitutes a region of instability for adenoviral vectors. CMV without intron A has been found to effectuate (Examples 1-3) comparable expression capabilities in vitro when driving HIV gag expression and, furthermore, behaved equivalently to intron A-containing constructs in Balb/c mice in vivo with respect to their antibody and T-cell responses at both dosages of plasmid DNA tested (20 µg and 200 µg). Those skilled in the art will appreciate that any of a number of other known promoters, such as the strong immunoglobulin, or other eukaryotic gene promoters may also be used, including the EF1 alpha promoter, the murine CMV promoter, Rous sarcoma virus (RSV) promoter, SV40 early/late promoters and the beta-actin promoter.

5

10

15

20

25

30

35

In preferred embodiments, the promoter may also comprise a regulatable sequence such as the Tet operator sequence. This would be extremely useful, for example, in cases where the gene products are effecting a result other than that desired and repression is sought.

The combination of the CMV promoter (devoid of the intron A region) with the BGH terminator is particularly preferred although other promoter/terminator combinations in the context of FG adenovirus may also be used.

Other embodiments incorporate a leader or signal peptide into the transgene. A preferred leader is that from the tissue-specific plasminogen activator protein, tPA. Examples include but are not limited to the various tPA-gag, tPA-pol and tPA-nef adenovirus-based vaccines disclosed throughout this specification.

In view of the improved adenovirus vectors described herein, an essential portion of the present invention are adenoviral-based HIV vaccines comprising said adenovirus backbones which may be administered to a mammalian host, preferably a human host, in either a prophylactic or therapeutic setting. The HIV vaccines of the present invention, whether administered alone or in combination regimens with other viral- or non-viral-based DNA vaccines, should elicit potent and broad cellular immune responses against HIV that will either lessen the likelihood of persistent virus infection and/or lead to the establishment of a clinically significant lowered virus load

subject to HIV infection or in combination with HAART therapy, mitigate the effects of previously established HIV infection (antiviral immunotherapy(ARI)). While any HIV antigen (e.g., gag, pol, nef, gp160, gp41, gp120, tat, rev, etc.) may be utilized in the herein described recombinant adenoviral vectors, preferred embodiments include the codon optimized p55 gag antigen (herein exemplified as MRKAd5gag), pol and nef. Sequences based on different Clades of HIV-1 are suitable for use in the instant invention, most preferred of which are Clade B and Clade C. Particularly preferred embodiments are those sequences (especially, codon-optimized sequences) based on concensus Clade B sequences. Preferred versions of the MRKAd5pol and MRKAd5nef series of adenoviral vaccines will encode modified versions of pol or nef, as discussed herein. Preferred embodiments of the MRKAd5HIV-1 vectors carrying HIV envelope genes and modifications thereof comprise the HIV codon-optimized env sequences of PCT International Applications PCT/US97/02294 and PCT/US97/10517, published August 28, 1997 (WO 97/31115) and December 24, 1997, respectively; both documents of which are hereby incorporated by reference.

5

10

15

20

25

30

35

A most preferred aspect of the instant invention is the disclosed use of the adenoviral vector described above to effectuate expression of HIV gag. Sequences for many genes of many HIV strains are publicly available in GENBANK and primary, field isolates of HIV are available from the National Institute of Allergy and Infectious Diseases (NIAID) which has contracted with Quality Biological (Gaithersburg, MD) to make these strains available. Strains are also available from the World Health Organization (WHO), Geneva Switzerland. It is preferred that the gag gene be from an HIV-1 strain (CAM-1; Myers et al, eds. "Human Retroviruses and AIDS: 1995, IIA3-IIA19, which is hereby incorporated by reference). This gene closely resembles the consensus amino acid sequence for the clade B (North American/European) sequence. Therefore, it is within the purview of the skilled artisan to choose an appropriate nucleotide sequence which encodes a specific HIV gag antigen, or immunologically relevant portion thereof. As shown in Example 25, a clade B or clade C based p55 gag antigen will potentially be useful on a global scale. As noted herein, the transgene of choice for insertion in to a DNA or MRKAd-based adenoviral vector of the present invention is a codon optimized version of p55 gag. Such a MRKAd5gag adenoviral vector is documented in Example 11 and is at least referred to herein as MRKAd5HIV-1gag. Of course, additional versions are contemplated, including but not limited to modifications such as promoter (e.g., mCMV for hCMV) and/or pA-terminations signal (SPA for bGH) switching, as well as generating MRK Ad5 backbones with or without deletion of the Ad5 E3 gene.

The present invention also relates a series of MRKAd5pol-based adenoviral vaccines which are shown herein to generate cellular immune responses subsequent to administration in mice and non-human primate studies. Several of the MRKAd5pol series are exemplified herein. One such adenoviral vector is referred to as MRKAd5hCMV-inact opt pol(E3+), which comprises the MRKAd5 backbone, the 5 hCMV promoter (no intron A), an inactivated pol transgene, and contains the Ad5 E3 gene in the adenoviral backbone. A second exemplified pre-adenovirus plasmid and concomitant virus is referred to as MRKAd5hCMV-inact opt pol(E3-), which is identical to the former adenoviral vector except that the E3 is deleted. Both constructions contain a codon optimized, inactivated version of HIV-1 Pol, wherein at 10 least the entire coding region is disclosed herein as SEQ ID NO:3 and the expressed protein is shown as SEQ ID NO:4 (see also Figure 17A-C and Table 1, which show targeted deletion for inactivated pol. This and other preferred codon optimized versions of HIV Pol as disclosed herein are essentially as described in U.S. Application Serial No. 09/745,221, filed December 21, 2000 and PCT International 15 Application PCT/US00/34724, also filed December 21, 2000, both documents which are hereby incorporated by reference. As disclosed in the above-mentioned documents, the open reading frame for these codon-optimized HIV-1 Pol-based DNA vaccines are represented by codon optimized DNA molecules encoding codon optimized HIV-1 Pol (e.g. SEQ ID NO:2), codon optimized HIV-1 Pol fused to an 20 amino terminal localized leader sequence (e.g. SEQ ID NO:6), and especially preferable, and exemplified by the MRKAd5-Pol construct in e.g., Example 19, biologically inactivated pol ("inact opt Pol"; e.g., SEQ ID NO:4) which is devoid of significant PR, RT, RNase or IN activity associated with wild type Pol. In addition, a construct related to SEO ID NO:4 is contemplated which contains a leader peptide at 25 the amino terminal region of the IA Pol protein. A specific construct is ligated within an appropriate DNA plasmid vector containing regulatory regions operatively linked to the respective HIV-1 Pol coding region, with or without a nucleotide sequence encoding a functional leader peptide. To this end, various HIV-1 Pol constructs disclosed herein relate to open reading frames for cloning to the enhanced first 30 generation Ad vectors of the present invention (such a series of MRKAd5pol adenoviral vaccine vectors), including but not limited to wild type Pol (comprising the DNA molecule encoding WT opt Pol, as set forth in SEQ ID NO:2), tPA-opt WTPol, (comprising the DNA molecule encoding tPA Pol, as set forth in SEQ ID NO:6), inact opt Pol (comprising the DNA molecule encoding IA Pol, as set forth in SEQ ID 35 NO:4), and tPA-inact opt Pol, (comprising the DNA molecule encoding tPA-inact opt

Pol, as set forth in SEQ ID NO:8). The pol-based versions of enhanced first generation adenovirus vaccines elicit CTL and Th cellular immune responses upon administration to the host, including primates and especially humans. As noted in the above, an effect of the cellular immune-directed vaccines of the present invention should be a lower transmission rate to previously uninfected individuals and/or reduction in the levels of the viral loads within an infected individual, so as to prolong the asymptomatic phase of HIV-1 infection.

5

The present invention further relates to a series of MRKAd5nef-based adenoviral vaccines which, similar to HIV gag and pol antigens, generate cellular immune responses subsequent to administration in mice and non-human primate 10 studies. The MRKAd5nef series are exemplified herein by utilizing the improved MRK adenoviral backbone in combination with modified versions of HIV nef. These exemplified MRKAd5nef vectors are as follows: (1) MRKAd5hCMVnef(G2A,LLAA) (E3+), which comprises the improved MRKAd5 backbone, a human CMV promoter an intact Ad5 E3 gene and a modified nef gene: (2) MRKAd5mCMV-15 nef(G2A,LLAA) (E3+), which is the same as (1) above but substituting a murine CMV promoter for a human CMV promoter; and (3) MRKAd5mCMV-tpanef(LLAA) (E3+), which is the same as (2) except that the nef transgene is tpanef(LLAA). Codon optimized versions of HIV-1 Nef and HIV-1 Nef modifications are essentially as described in U.S. Application Serial No. 09/738,782, filed December 15, 2000 and 20 PCT International Application PCT/US00/34162, also filed December 15, 2000, both documents which are hereby incorporated by reference. Particular embodiments of codon optimized Nef and Nef modifications relate to a DNA molecule encoding HIV-1 Nef from the HIV-1 ifrl isolate wherein the codons are optimized for expression in a mammalian system such as a human. The DNA molecule which encodes this protein 25 is disclosed herein as SEQ ID NO:9, while the expressed open reading frame is disclosed herein as SEQ ID NO:10. Another embodiment of Nef-based coding regions for use in the adenoviral vectors of the present invention comprise a codon optimized DNA molecule encoding a protein containing the human plasminogen activator (tpa) leader peptide fused with the NH2-terminus of the HIV-1 Nef 30 polypeptide. The DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:11, while the expressed open reading frame is disclosed herein as SEQ ID NO:12. Another modified Nef optimized coding region relates to a DNA molecule encoding optimized HIV-1 Nef wherein the open reading frame codes for modifications at the amino terminal myristylation site (Gly-2 to Ala-2) and 35 substitution of the Leu-174-Leu-175 dileucine motif to Ala-174-Ala-175, herein

described as opt nef (G2A, LLAA). The DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:13, while the expressed open reading frame is disclosed herein as SEQ ID NO:14. MRKAd5nef vectors (1) MRKAd5hCMV-nef(G2A,LLAA) (E3+) and (2) MRKAd5mCMV-nef(G2A,LLAA) (E3+) contain this transgene. An additional embodiment relates to a DNA molecule encoding optimized HIV-1 Nef wherein the amino terminal myristylation site and dileucine motif have been deleted, as well as comprising a tPA leader peptide. This DNA molecule, opt tpanef (LLAA), comprises an open reading frame which encodes a Nef protein containing a tPA leader sequence fused to amino acid residue 6-216 of HIV-1 Nef (jfrl), wherein Leu-174 and Leu-175 are substituted with Ala-174 and Ala-175, herein referred to as opt tpanef (LLAA) is disclosed herein as SEQ ID NO:15, while the expressed open reading frame is disclosed herein as SEQ ID NO:16. The MRKAd5nef vector "MRKAd5mCMV-tpanef(LLAA) (E3+)" contains this transgene.

5

10

15

20

25

30

35

Along with the improved MRKAd5gag adenovirus vaccine vector described herein, generation of a MRKAd5pol and MRKAd5nef adenovirus vector provide for enhanced HIV vaccine capabilities. Namely, the generation of this trio of adenoviral vaccine vectors, all shown to generate effective cellular immune responses subsequent to host administration, provide for the ability to administer these vaccine candidates not only alone, but preferably as part of a divalent (i.e., gag and nef, gag and pol, or pol and nef components) or a trivalent vaccine (i.e., gag, pol and nef components). Therefore, a preferred aspect of the present invention are vaccine formulations and associated methods of administration and concomitant generation of host cellular immune responses associated with formulating three separate series of MRKAd5based adenoviral vector vaccines. Of course, this MRKAd5 vaccine series based on distinct HIV antigens promotes expanded opportunities for formulation of a divalent or trivalent vaccine, or possibly administration of separate formulations of one or more monovalent or divalent formulations within a reasonable window of time. It is also within the scope of the present invention to embark on combined modality regimes which include multiple but distinct components from a specific antigen. An example, but certainly not a limitation, would be separate MRKAd5pol vectors, with one vaccine vector expressing wild type Pol (SEQ ID NO:2) and another MRKAd5pol vector expressing inactivated Pol (SEQ ID NO:6). Another example might be separate MRKAd5nef vectors, with one vaccine vector expressing the tPA/LLAA version of Nef (SEQ ID NO:16) and another MRKAd5nef vector expressing the G2A,LLAA modified version of Nef (SEQ ID NO:14). Therefore, the MRKAd5 adenoviral vectors of the present invention may be used in combination

with multiple, distinct HIV antigen classes. Each HIV antigen class is subject to sequence manipulation, thus providing for a multitude of potential vaccine combinations; and such combinations are within the scope of the present invention. The utilization of such combined modalities vaccine formulation and administration increase the probability of eliciting an even more potent cellular immune response when compared to inoculation with a single modality regimen.

5

10

15

20

25

30

35

The present invention also relates to application of a mono-, dual-, or trimodality administration regime of the MRKAd5gag, pol and nef adenoviral vaccine series in a prime/boost vaccination schedule. This prime/boost schedule may include any reasonable combination of the MRKAd5gag, pol and nef adenoviral vaccine series disclosed herein. In addition, a prime/boost regime may also involve other viral and/or non-viral DNA vaccines. A preferable addition to an adenoviral vaccine vector regime includes but is not limited to plasmid DNA vaccines, especially DNA plasmid vaccines that contain at least one of the codon optimized gag, pol and nef constructions, as disclosed herein.

Therefore, one aspect of this invention is the administration of the adenoviral vector containing the optimized gag gene in a prime/boost regiment in conjunction with a plasmid DNA encoding gag. To distinguish this plasmid from the adenoviralcontaining shuttle plasmids used in the construction of an adenovirus vector, this plasmid will be referred to as a "vaccine plasmid" or "DNA plasmid vaccine". Preferred vaccine plasmids for use in this administration protocol are disclosed in pending U.S. patent application 09/017,981, filed February 3, 1998 and WO98/34640, published August 13, 1998, both of which are hereby incorporated by reference. Briefly, the preferred vaccine plasmid is designated V1Jns-FLgag, which expresses the same codon-optimized gag gene as the adenoviral vectors of this invention (see Figure 2 for the nucleotide sequence of the exemplified optimized codon version of full length p55 gag). The vaccine plasmid backbone, designated V1Jns contains the CMV immediate-early (IE) promoter and intron A, a bovine growth hormone-derived polyadenylation and transcription termination sequence as the gene expression regulatory elements, and a minimal pUC backbone; see Montgomery et al., 1993, DNA Cell Biol. 12:777-783. The pUC sequence permits high levels of plasmid production in E. coli and has a neomycin resistance gene in place of an ampicillin resistance gene to provide selected growth in the presence of kanamycin. Alternatively, a vaccine plasmid which has the CMV promoter deleted of intron A can be used. Those of skill in the art will recognize that alternative vaccine plasmid

vectors may be easily substituted for these specific constructs, and this invention specifically envisions use of such alternative plasmid DNA vaccine vectors.

5

10

15

20

25

30

35

Another aspect of the present invention is a prime/boost regimen which includes a vaccine plasmid which encodes an HIV pol antigen, preferably a codon optimized form of pol and also preferably a vaccine plasmid which comprises a nucleotide sequence which encodes a Pol antigen selected from the group of Pol antigens as shown in SEQ ID NOs: 2, 4, 6 and 8. The variety of potential DNA plasmid vaccines which encode various biologically active forms of HIV-1 Pol, wherein administration, intracellular delivery and expression of the HIV-1 Pol gene of interest elicits a host CTL and Th response. The preferred synthetic DNA molecules of the present invention encode codon optimized wild type Pol (without Pro activity) and various codon optimized inactivated HIV-1 Pol proteins. The HIV-1 pol open reading disclosed herein are especially preferred for pharmaceutical uses, especially for human administration as delivered via a recombinant adenoviral vaccine, especially an enhanced first generation recombinant adenoviral vaccine as described herein. Several embodiments of this portion of the invention are provided in detail below, namely DNA molecules which comprise a HIV-1 pol open reading frame, whether encoding full length pol or a modification or fusion as described herein. wherein the codon usage has been optimized for expression in a mammal, especially a human. Again, these DNA sequences are positioned appropriately within a recombinant adenoviral vector, such as the exemplified recombinant adenoviral vector described herein, so as to promote expression of the respective HIV-1 Pol gene of interest, and subsequent to administration, elicit a host CTL and Th response. Again, these preferred, but in no way limiting, pol genes are as disclosed herein and essentially as described in U.S. Application Serial No. 09/745,221, filed December 21, 2000 and PCT International Application PCT/US00/34724, also filed December 21, 2000, both documents which are hereby incorporated by reference.

A third series of vaccine plasmids which are useful in a combined modality and/or prime/boost regimen are vaccine plasmids which encode an HIV nef antigen or biologically and/or immunologically relevant modification thereof. As noted elsewhere, preferred vaccine plasmids contain a codon optimized form of nef and also preferably comprise a nucleotide sequence which encodes a Nef antigen selected from the group of Nef antigens as shown in SEQ ID NOs: 10, 12, 14 and 16. These preferred nef coding regions are disclosed herein, as well as being described in U.S. Application Serial No. 09/738,782, filed December 15, 2000 and PCT International

Application PCT/US00/34162, also filed December 15, 2000, both documents which are hereby incorporated by reference.

5

10

15

Therefore, the adenoviral vaccines and plasmid DNA vaccines of this invention may be administered alone, or may be part of a prime and boost administration regimen. A mixed modality priming and booster inoculation scheme will result in an enhanced immune response, particularly is pre-existing anti-vector immune responses are present. This one aspect of this invention is a method of priming a subject with the plasmid vaccine by administering the plasmid vaccine at least one time, allowing a predetermined length of time to pass, and then boosting by administering the adenoviral vaccine. Multiple primings typically, 1-4, are usually employed, although more may be used. The length of time between priming and boost may typically vary from about four months to a year, but other time frames may be used. In experiments with rhesus monkeys, the animals were primed four times with plasmid vaccines, then were boosted 4 months later with the adenoviral vaccine. Their cellular immune response was notably higher than that of animals which had only received adenoviral vaccine. The use of a priming regimen may be particularly preferred in situations where a person has a pre-existing anti-adenovirus immune response.

Furthermore and in the alternative, multiple HIV-1 viral antigens, such as the MRKAd5 adenoviral vaccines disclosed herein, may be ligated into a proper shuttle 20 plasmid for generation of a pre-adenoviral plasmid comprising multiple open reading frames. For example a trivalent vector may comprise a gag-pol-nef fusion, in either a E3(-) or E3(+) background, preferably a E3 deleted backbone, or possible a "2+1" divalent vaccine, such as a gag-pol fusion (i.e., codon optimized p55 gag and inactivated optimized pol; Example 29 and Table 25) within the same MRKAd5 25 backbone, with each open reading frame being operatively linked to a distinct promoter and transcription termination sequence. Alternatively, the two open reading frames may be operatively linked to a single promoter, with the open reading frames operatively linked by an internal ribosome entry sequence (IRES), as disclosed in International Publication No. WO 95/24485, which is hereby incorporated by 30 reference. Figure 9 shows that the use of multiple promoters and termination sequences provide for similar growth properties, while Figure 28 shows that these MRKAd5gag-based vectors are also stable at least through passage 21. In the absence of the use of IRES-based technology, it is preferred that a distinct promoter be used to support each respective open reading frame, so as to best preserve vector stability. As 35 examples, and certainly not as limitations, potential multiple transgene vaccines may

include a three transgene vector such as hCMV-gagpol-bGHpA + mCMV-nef-SPA in an E3 deleted backbone or hCMV-gagpol-bGHpA + mCMV-nef-SPA(E3+). Potential "2+1" divalent vaccines of the present invention might be a hCMV-gagbGHpA + mCMV-nef-SPA in an E3+ backbone (vector #1) in combination with hCMV-pol-bGHpA in an E3+ backbone (vector #2), with all transgenes in the E1 parallel orientation. Fusion constructs other than the gag-pol fusion described above are also suitable for use in various divalent vaccine strategies and can be composed of any two HIV antigens fused to one another (e.g.,, nef-pol and gag-nef). These adenoviral compositions are, as above, preferably delivered along with an adenoviral composition comprising an additional HIV antigen in order to diversify the immune response generated upon administration. Therefore, a multivalent vaccine delivered in a single, or possible second, adenoviral vector is certainly contemplated as part of the present invention. Again, this mode of administration is another example of whereby an efficaceous adenovirus-based HIV-1 vaccine may be administered via a combined modality regime. It is important to note, however, that in terms of deciding on an insert for the disclosed adenoviral vectors, due consideration must be dedicated to the effective packaging limitations of the adenovirus vehicle. Adenovirus has been shown to exhibit an upper cloning capacity limit of approximately 105% of the wildtype Ad5 sequence.

5

10

15

20

30

35

Regardless of the gene chosen for expression, it is preferred that the sequence be "optimized" for expression in a human cellular environment. A "triplet" codon of four possible nucleotide bases can exist in 64 variant forms. That these forms provide the message for only 20 different amino acids (as well as transcription initiation and termination) means that some amino acids can be coded for by more than one codon. Indeed, some amino acids have as many as six "redundant", alternative codons while some others have a single, required codon. For reasons not completely understood, alternative codons are not at all uniformly present in the endogenous DNA of differing types of cells and there appears to exist variable natural hierarchy or "preference" for certain codons in certain types of cells. As one example, the amino acid leucine is specified by any of six DNA codons including CTA, CTC, CTG, CTT, TTA, and TTG (which correspond, respectively, to the mRNA codons, CUA, CUC. CUG, CUU, UUA and UUG). Exhaustive analysis of genome codon frequencies for microorganisms has revealed endogenous DNA of E. coli most commonly contains the CTG leucine-specifying codon, while the DNA of yeasts and slime molds most commonly includes a TTA leucine-specifying codon. In view of this hierarchy, it is generally held that the likelihood of obtaining high levels of expression of a leucine-

rich polypeptide by an *E. coli* host will depend to some extent on the frequency of codon use. For example, a gene rich in TTA codons will in all probability be poorly expressed in *E. coli*, whereas a CTG rich gene will probably highly express the polypeptide. Similarly, when yeast cells are the projected transformation host cells for expression of a leucine-rich polypeptide, a preferred codon for use in an inserted DNA would be TTA.

5

10

15

20

25

30

35

The implications of codon preference phenomena on recombinant DNA techniques are manifest, and the phenomenon may serve to explain many prior failures to achieve high expression levels of exogenous genes in successfully transformed host organisms—a less "preferred" codon may be repeatedly present in the inserted gene and the host cell machinery for expression may not operate as efficiently. This phenomenon suggests that synthetic genes which have been designed to include a projected host cell's preferred codons provide a preferred form of foreign genetic material for practice of recombinant DNA techniques. Thus, one aspect of this invention is an adenovirus vector or adenovirus vector in some combination with a vaccine plasmid where both specifically include a gene which is codon optimized for expression in a human cellular environment. As noted herein, a preferred gene for use in the instant invention is a codon-optimized HIV gene and, particularly, HIV gag, pol or nef.

Adenoviral vectors in accordance with the instant invention can be constructed using known techniques, such as those reviewed in Hitt et al, 1997 "Human Adenovirus Vectors for Gene Transfer into Mammalian Cells" Advances in Pharmacology 40:137-206, which is hereby incorporated by reference.

In constructing the adenoviral vectors of this invention, it is often convenient to insert them into a plasmid or shuttle vector. These techniques are known and described in Hitt et al., *supra*. This invention specifically includes both the adenovirus and the adenovirus when inserted into a shuttle plasmid.

Preferred shuttle vectors contain an adenoviral portion and a plasmid portion. The adenoviral portion is essentially the same as the adenovirus vector discussed supra, containing adenoviral sequences (with non-functional or deleted E1 and E3 regions) and the gene expression cassette, flanked by convenient restriction sites. The plasmid portion of the shuttle vector often contains an antibiotic resistance marker under transcriptional control of a prokaryotic promoter so that expression of the antibiotic does not occur in eukaryotic cells. Ampicillin resistance genes, neomycin resistance genes and other pharmaceutically acceptable antibiotic resistance markers may be used. To aid in the high level production of the polynucleotide by

fermentation in prokaryotic organisms, it is advantageous for the shuttle vector to contain a prokaryotic origin of replication and be of high copy number. A number of commercially available prokaryotic cloning vectors provide these benefits. It is desirable to remove non-essential DNA sequences. It is also desirable that the vectors not be able to replicate in eukaryotic cells. This minimizes the risk of integration of polynucleotide vaccine sequences into the recipients' genome. Tissue-specific promoters or enhancers may be used whenever it is desirable to limit expression of the polynucleotide to a particular tissue type.

In one embodiment of this invention, the pre-plasmids (e.g., pMRKAd5pol, pMRKAd5nef and pMRKAd5gag were generated by homologous recombination using the MRKHVE3 (and MRKHVO for the E3- version) backbones and the appropriate shuttle vector, as shown for pMRKAd5pol in Figure 22 and for pMRKAd5nef in Figure 23. The plasmid in linear form is capable of replication after entering the PER.C6[®] cells and virus is produced. The infected cells and media were harvested after viral replication was complete.

Viral vectors can be propagated in various E1 complementing cell lines, including the known cell lines 293 and PER.C6[®]. Both these cell lines express the adenoviral E1 gene product. PER.C6[®] is described in WO 97/00326 (published January 3, 1997) and issued U.S. Patent No. 6,033,908, both of which are hereby incorporated by reference. It is a primary human retinoblast cell line transduced with an E1 gene segment that complements the production of replication deficient (FG) adenovirus, but is designed to prevent generation of replication competent adenovirus by homologous recombination. Cells of particular interest have been stably transformed with a transgene that encodes the AD5E1A and E1B gene, like PER.C6[®], from 459 bp to 3510 bp inclusive. 293 cells are described in Graham et al., 1977 J. Gen. Virol 36:59-72, which is hereby incorporated by reference. As stated above, consideration must be given to the adenoviral sequences present in the complementing cell line used. It is important that the sequences not overlap with that present in the vector if the possibility of recombination is to be minimized.

It has been found that vectors generated in accordance with the above description are more effective in inducing an immune response and, thus, constitute very promising vaccine candidates. More particularly, it has been found that first generation adenoviral vectors in accordance with the above description carrying a codon-optimized HIV gag gene, regulated with a strong heterologous promoter can be used as human anti-HIV vaccines, and are capable of inducing immune responses.

Standard techniques of molecular biology for preparing and purifying DNA constructs enable the preparation of the DNA immunogens of this invention.

A vaccine composition comprising an adenoviral vector in accordance with the instant invention may contain physiologically acceptable components, such as buffer, normal saline or phosphate buffered saline, sucrose, other salts and polysorbate. One preferred formulation has: 2.5-10 mM TRIS buffer, preferably about 5 mM TRIS buffer; 25-100 mM NaCl, preferably about 75 mM NaCl; 2.5-10% sucrose, preferably about 5% sucrose; 0.01 -2 mM MgCl₂; and 0.001%-0.01% polysorbate 80 (plant derived). The pH should range from about 7.0-9.0, preferably about 8.0. One skilled in the art will appreciate that other conventional vaccine excipients may also be used it make the formulation. The preferred formulation contains 5mM TRIS, 75 mM NaCl, 5% sucrose, 1mM MgCl₂, 0.005% polysorbate 80 at pH 8.0 This has a pH and divalent cation composition which is near the optimum for Ad5 stability and minimizes the potential for adsorption of virus to a glass surface. It does not cause tissue irritation upon intramuscular injection. It is preferably frozen until use.

The amount of adenoviral particles in the vaccine composition to be introduced into a vaccine recipient will depend on the strength of the transcriptional and translational promoters used and on the immunogenicity of the expressed gene product. In general, an immunologically or prophylactically effective dose of 1×10^7 to 1×10^{12} particles and preferably about 1×10^{10} to 1×10^{11} particles is administered directly into muscle tissue. Subcutaneous injection, intradermal introduction, impression through the skin, and other modes of administration such as intraperitoneal, intravenous, or inhalation delivery are also contemplated. It is also contemplated that booster vaccinations are to be provided. Following vaccination with HIV adenoviral vector, boosting with a subsequent HIV adenoviral vector and/or plasmid may be desirable. Parenteral administration, such as intravenous, intramuscular, subcutaneous or other means of administration of interleukin-12 protein, concurrently with or subsequent to parenteral introduction of the vaccine compositions of this invention is also advantageous.

The adenoviral vector and/or vaccine plasmids of this invention polynucleotide may be unassociated with any proteins, adjuvants or other agents which impact on the recipients' immune system. In this case, it is desirable for the vector to be in a physiologically acceptable solution, such as, but not limited to, sterile saline or sterile buffered saline. Alternatively, the vector may be associated with an adjuvant known in the art to boost immune responses (i.e., a "biologically effective"

adjuvant), such as a protein or other carrier. Vaccine plasmids of this invention may, for instance, be delivered in saline (e.g., PBS) with or without an adjuvant. Preferred adjuvants are Alum or CRL1005 Block Copolymer. Agents which assist in the cellular uptake of DNA, such as, but not limited to, calcium ions, may also be used to advantage. These agents are generally referred to herein as transfection facilitating reagents and pharmaceutically acceptable carriers. Techniques for coating microprojectiles coated with polynucleotide are known in the art and are also useful in connection with this invention.

5

10

15

20

25

30

35

This invention also includes a prime and boost regimen wherein a first adenoviral vector is administered, then a booster dose is given. The booster dose may be repeated at selected time intervals. Alternatively, a preferred inoculation scheme comprises priming with a first adenovirus serotype and then boosting with a second adenovirus serotype. More preferably, the inoculation scheme comprises priming with a first adenovirus serotype and then boosting with a second adenovirus serotype, wherein the first and second adenovirus serotypes are classified within separate subgroups of adenoviruses. The above prime/boost schemes are particularly preferred in those situations where a preexisting immunity is identified to the adenoviral vector of choice. In this type of scheme, the individual or population of individuals is primed with an adenovirus of a serotype other than that to which the preexisting immunity is identified. This enables the first adenovirus to effectuate sufficient expression of the transgene while evading existing immunity to the second adenovirus (the boosting adenovirus) and, further, allows for the subsequent delivery of the transgene via the boosting adenovirus to be more effective. Adenovirus serotype 5 is one example of a virus to which such a scheme might be desirable. In accordance with this invention, therefore, one might decide to prime with a non-group C adenovirus (e.g., Ad12, a group A adenovirus, Ad24, a group D adenovirus, or Ad35, a group B adenovirus) to evade anti-Ad5 immunity and then boost with Ad5, a group C adenovirus. Another preferred embodiment involves administration of a different adenovirus (including non-human adenovirus) vaccine followed by administration of the adenoviral vaccines disclosed. In the alternative, a viral antigen of interest can be first delivered via a viral vaccine other than an adenovirus-based vaccine, and then followed with the adenoviral vaccine disclosed. Alternative viral vaccines include but are not limited to pox virus and venezuelan equine encephilitis virus.

A large body of human and animal data supports the importance of cellular immune responses, especially CTL in controlling (or eliminating) HIV infection. In humans, very high levels of CTL develop following primary infection and correlate

with the control of viremia. Several small groups of individuals have been described who are repeatedly exposed to HIV by remain uninfected; CTL has been noted in several of these cohorts. In the SIV model of HIV infection, CTL similarly develops following primary infection, and it has been demonstrated that addition of anti-CD8 monoclonal antibody abrogated this control of infection and leads to disease progression. This invention uses adenoviral vaccines alone or in combination with plasmid vaccines to induce CTL.

The following non-limiting Examples are presented to better illustrate the invention.

10

15

20

25

30

35

5

EXAMPLE 1

Removal of the Intron A Portion of the hCMV Promoter GMP grade pVIInsHIV gag was used as the starting material to amplify the hCMV promoter. PVIInsHIVgag is a plasmid comprising the CMV immediate-early (IE) promoter and intron A, a full-length codon-optimized HIV gag gene, a bovine growth hormone-derived polyadenylation and transcriptional termination sequence, and a minimal pUC backbone; see Montgomery et al., supra for a description of the plasmid backbone. The amplification was performed with primers suitably positioned to flank the hCMV promoter. A 5' primer was placed upstream of the Msc1 site of the hCMV promoter and a 3' primer (designed to contain the BgIII recognition sequence) was placed 3' of the hCMV promoter. The resulting PCR product (using high fidelity Taq polymerase) which encompassed the entire hCMV promoter (minus intron A) was cloned into TOPO PCR blunt vector and then removed by double digestion with Msc1 and BgIII. This fragment was then cloned back into the original GMP grade pV1InsHIVgag plasmid from which the original promoter, intron A, and the gag gene were removed following Msc1 and BgIII digestion. This ligation reaction resulted in the construction of a hCMV promoter (minus intron A) + bGHpA expression cassette within the original pV1JnsHIVgag vector backbone. This vector is designated pVIInsCMV(no intron).

The FLgag gene was excised from pV1JnsHIVgag using BgIII digestion and the 1,526 bp gene was gel purified and cloned into pV1JnsCMV(no intron) at the BgIII site. Colonies were screened using Sma1 restriction enzymes to identify clones that carried the Flgag gene in the correct orientation. This plasmid, designated pV1JnsCMV(no intron)-FLgag-bGHpA, was fully sequenced to confirm sequence integrity.

Two additional transgenes were also constructed. The plasmid, pV1JnsCMV(no intron)-FLgag-SPA, is identical to pV1JnsCMV(no intron)-FLgag-bGHpA except that the bovine growth hormone polyadenylation signal has been replaced with a short synthetic polyA signal (SPA) of 50 nucleotides in length. The sequence of the SPA is as shown, with the essential components (poly(A) site, (GT)_n, and (T)_n; respectively) underlined:

<u>AATAAA</u>AGATCTTTATTTTCATTAGATCT<u>GTGTG TTGGTTTTTTGTGTG</u> (SEQ ID NO:18).

The plasmid, pV1Jns-mCMV-FLgag-bGHpA, is identical to the pV1JnsCMV(no intron)-FLgag-bGHpA except that the hCMV promoter has been removed and replaced with the murine CMV (mCMV) promoter.

Figure 3 diagrammatically shows the new transgene constructs in comparison with the original transgene.

15 EXAMPLE 2

5

10

20

25

Gag Expression Assay for Modified Gag Transgenes

Gag Elisa was performed on culture supernatants obtained from transient tissue culture transfection experiments in which the two new hCMV-containing plasmid constructs, pV1JnsCMV(no intron)-FLgag-bGHpA and pV1JnsCMV(no intron)-FLgag-SPA, both devoid of intron A, were compared to pV1JnsHIVgag which, as noted above possesses the intron A as part of the hCMV promoter. Table 2 below shows the *in vitro* gag expression data of the new gag plasmids compared with the GMP grade original plasmid. The results displayed in Table 2 show that both of the new hCMV gag plasmid constructs have expression capacities comparable to the original plasmid construct which contains the intron A portion of the hCMV promoter.

Table 2: In vitro DNA transfection of original and new plasmid HIV-1 gag constructs.

Plasmid	μg gag/10e6 COS cells/5μg DNA/48 hr
HIVFL-gagPR9901ª	10.8
PVIIns-hCMV-FLgag-bGHpAb	16.6
pV1Jns-hCMV-FLgag-SPAbc	12.0

^a GMP grade pV1Jns-hCMVintronA-FLgag-bGHpA.

10

15

20

EXAMPLE 3

Rodent (Balb/c) Study for Modified gag Transgenes
A rodent study was performed on the two new plasmid constructs
described above – pV1JnsCMV(no intron)-FLgag-bGHpA and pV1JnsCMV(no
intron)-FLgag-SPA - in order to compare them with the construct described above
possessing the intron A portion of the CMV promoter, pV1JnsHIVgag. Gag antibody
and Elispot responses (described in PCT International Application No.
PCT/US00/18332 (WO 01/02607) filed July 3, 2000, claiming priority to U.S.
Provisional Application Serial No. 60/142,631, filed July 6, 1999 and U.S.
Application Serial No. 60/148,981, filed August 13, 1999, all three applications which
are hereby incorporated by reference) were measured. The results displayed in Table
3 below, show that the new plasmid constructs behaved equivalently to the original
construct in Balb/c mice with respect to their antibody and T-cell responses at both
dosages of plasmid DNA tested, 20 μg and 200 μg.

^b New plasmid constructions that have the intron A portion removed from the hCMV promoter.

^c In this construct the bGH terminator has been replaced with the short synthetic polyadenylation signal (SPA)

EXAMPLE 4

Table 3: HIV191: Immunogenicity of V1Jns-gag under different promoter and termination control elements.

DNA*	Dose, ug ^b		Anti-p24 Titers (3 Wk PD1)°		SFC/10^6 Cells (4 Wk PD1) ^d			
Promoter/terminator		GMT	+SE	-SE	Media	gag197-205	p24	
H!VFL-gagPR9901	200	12800	4652	3412	2(2)	129(19)	30(11)	
(GMP grade)	20	5572	1574	1227	0	56(9)	25(6)	
pV1Jns-hCMV-	200	11143	2831	2257	0	98(5)	12(6)	
FL-gag-bGHpA	20	7352	2808	2032	0	73(9)	11(6)	
pV1Jns-hCMV-	200	16890	5815	4326	1(1)	94(4)	26(7)	
FL-gag-SPA	20	5971	5361	2825	0	85(17)	38(10)	
Naïve	0	123	50	36	0	0	0	

in PBS

5

20

Construction of the Modified Shuttle Vector - "MRKpdelE1 Shuttle"

The modifications to the original Ad5 shuttle vector (pdelE1sp1A; a vector comprising Ad5 sequences from basepairs 1-341 and 3524-5798, with a multiple cloning region between nucleotides 341 and 3524 of Ad5, included the following three manipulations carried out in sequential cloning steps as follows:

- (1) The left ITR region was extended to include the *Pac1* site at the junction between the vector backbone and the adenovirus left ITR sequences. This allow for easier manipulations using the bacterial homologous recombination system.
- 10 (2) The packaging region was extended to include sequences of the wild-type (WT) adenovirus from 342 bp to 450 bp inclusive.
 - (3) The area downstream of pIX was extended 13 nucleotides (i.e., nucleotides 3511-3523 inclusive).
- These modifications (Figure 4) effectively reduced the size of the E1 deletion without overlapping with any part of the E1A/E1B gene present in the transformed PER.C6[®] cell line. All manipulations were performed by modifying the Ad shuttle vector pdelE1sp1A.

Once the modifications were made to the shuttle vector, the changes were incorporated into the original Ad5 adenovector backbones (pAdHVO and pAdHVE3) by bacterial homologous recombination using *E. coli* BJ5183 chemically competent cells.

bi.m. Injections into both quads, 50 µL per quad

on=10;GMT, geometric mean titer; SE, standard, error

dn=5, pooled spleens; mean of triplicate wells and standard, deviation, in parentheses;

EXAMPLE 5

Construction of Modified Adenovector Backbones (E3+ and E3-)

The original adenovectors pAdHVO (comprising all Ad5 sequences except those nucleotides encompassing the E1 and E3 regions) and pADHVE3 (comprising all Ad5 sequences except those nucleotides encompassing the E1 region), were each 5 reconstructed so that they contained the modifications to the E1 region. This was accomplished by digesting the newly modified shuttle vector (MRKpdelE1 shuttle) with Pac1 and BstZ1101 and isolating the 2,734 bp fragment which corresponds to the adenovirus sequence. This fragment was co-transformed with DNA from either Cla1 linearized pAdHVO (E3- adenovector) or Cla1 linearized pAdHVE3 10 (E3+adenovector) into E. coli BJ5183 competent cells. At least two colonies from each transformation were selected and grown in Terrific™ broth for 6-8 hours until turbidity was reached. DNA was extracted from each cell pellet and then transformed into E. coli XL1 competent cells. One colony from each transformation was selected and grown for plasmid DNA purification. The plasmid was analyzed by restriction 15 digestions to identify correct clones. The modified adenovectors were designated MRKpAdHVO (E3- plasmid) and MRKpAdHVE3 (E3+ plasmid). Virus from these new adenovectors (MRKHVO and MRKHVE3, respectively) as well as the old version of the adenovectors were generated in the PER.C6® cell lines to accommodate the following series of viral competition experiments. In addition, the multiple 20 cloning site of the original shuttle vector contained ClaI, BamHI, Xho I, EcoRV, HindIII, Sal I, and Bgl II sites. This MCS was replaced with a new MCS containing Not I. Cla I. EcoRV and Asc I sites. This new MCS has been transferred to the MRKpAdHVO and MRKpAdHVE3 pre-plasmids along with the modification made to the packaging region and pIX gene. 25

EXAMPLE 6

Analysis of the Effect of the Packaging Signal Extension

To study the effects of the modifications made to the E1 deletion region, the viruses obtained from the original backbone (pAdHVE3) and the new backbone (MRKpAdHVE3) were mixed together in equal MOI ratios (1:1 and 5:5) and passaged through several rounds; see Figure 5, Expt.#1. Both of the viruses in the experiment contained the E3 gene intact and did not contain a transgene. The only difference between the two viruses was within the region of the E1 deletion. Following the coinfection of the viruses at P1 (passage 1), the mixtures were propagated through an additional 4 passages at which time the cells were harvested

30

35

and the virus extracted and purified by CsCl banding. The viral DNA was extracted and digested with *Hind*III and the digestion products were then radioactively labeled. For the controls, the respective pre-plasmids (pAdHVE3 ("OLD E3+"); MRKpAdHVE3 ("NEW E3+")) were also digested with *Hind*III (and *Pac1* to remove the vector backbone) and subsequently labeled with [³³P]dATP. The radioactively labeled digestion products were subjected to gel electrophoresis and the gel was dried down onto Whatman paper before being exposed to autoradiographic film. Figure 6 clearly shows that the new adenovirus which has the addition made to the packaging signal region has a growth advantage compared with the original adenovirus. In the experiments performed (at either ratio tested), only the digestion bands pertaining to the newly modified virus were present. The diagnostic band of size 3,206 (from the new virus) was clearly present. However, there was no evidence of the diagnostic band of size 2,737 bp expected from the original virus.

15

20

25

30

35

10

5

EXAMPLE 7

Analysis of the Effect of the E3 Gene

The second set of the virus competition study involved mixing equal MOI ratio (1:1) of the newly modified viruses, that obtained from MRKpAdHVO and MRKpAdHVE3 (Figure 5, Expt. #2). In this set, both viruses had the new modifications made to the E1 deletion. The first virus (that from MRKpAdHVO) does not contain an E3 gene. The second virus (that from MRKpAdHVE3) does contain the E3 gene. Neither of the viruses contain a transgene. Following coinfection of the viruses, the mixtures were propagated through an additional 4 passages at which time the cells were harvested and the total virus extracted and purified by CsCl banding. The viral DNA was extracted and digested with *Hind*III and the digestion products were then radioactively labeled. For the controls, the respective pre-plasmids MRKpAdHVO ("NEW E3-"); MRKpAdHVE3 ("NEW E3+") were also digested with HindIII (and Pac1 to remove the vector backbone) and then labeled with [33P]dATP. The radioactively labeled digestion products were subjected to gel electrophoresis and the gel was dried down onto Whatman paper before being exposed to autoradiographic film. Figure 6 shows the results of the viral DNA analysis of the E3+ virus and E3- virus mixing experiment. The diagnostic band corresponding to the E3+ virus (5,665 bp) was present in greater amount compared with the diagnostic band of 3,010 bp corresponding to the E3- virus. This indicates that the virus that contains the E3 gene is able to amplify more rapidly

compared with the virus that does not contain an E3 gene. This increased amplification capacity has been confirmed by growth studies; see Table 4 below.

EXAMPLE 8

Construction of the new shuttle vector containing modified gag transgene – "MRKpdelE1-CMV(no intron)-FLgag-bGHpA"

The modified plasmid pV1JnsCMV(no intron)-FLgag-bGHpA was digested with Msc1 overnight and then digested with Sfi1 for 2 hours at 50°C. The DNA was then treated with Mungbean nuclease for 30 mins at 30°C. The DNA mixture was desalted using the Qiaex II kit and then Klenow treated for 30 mins at 37°C to fully blunt the ends of the transgene fragment. The 2,559 bp transgene fragment was then gel purified. The modified shuttle vector (MRKpdelE1 shuttle) was linearized by digestion with EcoRV, treated with calf intestinal phosphatase and the resulting 6,479 bp fragment was then gel purified. The two purified fragments were then ligated together and several dozen clones were screened to check for insertion of the transgene within the shuttle vector. Diagnostic restriction digestion was performed to identify those clones carrying the transgene in the E1 parallel and E1 anti-parallel orientation. This strategy was followed to clone in the other gag transgenes in the MRKpdelE1 shuttle vector.

20

25

30

35

5

10

15

EXAMPLE 9

Construction of the MRK FG Adenovectors

The shuttle vector containing the HIV-1 gag transgene in the E1 parallel orientation, MRKpdelE1-CMV(no intron)-FLgag-bGHpA, was digested with Pac1. The reaction mixture was digested with BsfZ171. The 5,291 bp fragment was purified by gel extraction. The MRKpAdHVE3 plasmid was digested with Cla1 overnight at 37°C and gel purified. About 100 ng of the 5,290 bp shuttle +transgene fragment and ~100 ng of linearized MRKpAdHVE3 DNA were co-transformed into E. coli BJ5183 chemically competent cells. Several clones were selected and grown in 2 ml Terrific™ broth for 6-8 hours, until turbidity was reached. The total DNA from the cell pellet was purified using Qiagen alkaline lysis and phenol chloroform method. The DNA was precipitated with isopropanol and resuspended in 20 μl dH₂0. A 2 μl aliquot of this DNA was transformed into E. coli XL-1 competent cells. A single colony from each separate transformation was selected and grown overnight in 3 ml LB +100 μg/ml ampicillin. The DNA was isolated using Qiagen columns. A positive clone was identified by digestion with the restriction enzyme BstEII which cleaves

within the gag gene as well as the plasmid backbone. The pre-plasmid clone is designated MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA and is 37,498 bp in size. This strategy was followed to generate E3- and E3+ versions of each of the other gag transgene constructions in both E1 parallel and E1 anti-parallel versions. Figures 7A, 7B and 7C show the various combinations of adenovectors constructed.

EXAMPLE 10

Plasmid Competition Studies

A series of plasmid competition studies was carried out. Briefly, the screening of the various combinations of new constructs was performed by mixing equal amounts of each of two competing plasmids. In the experiment shown in Figure 8A, plasmids containing the same transgene but in different orientations were mixed together to create a "competition" between the two plasmids. The aim was to look at the effects of transgene orientation. In the experiment shown in Figure 8B, plasmids containing different polyadenylation signals (but in the same orientation) were mixed together in equal amounts. The aim was to assess effects of polyA signals. Following the initial transfection, the virus was passaged through ten rounds and the viral DNA analyzed by radioactive restriction analysis.

Analysis of the viral species from the plasmid mixing experiment (Figure 8A) showed that adenovectors which had the transgene inserted in the E1 parallel orientation amplified better and were able to out-compete the adenovirus which had the transgene inserted in the E1 anti-parallel orientation. Viral DNA analysis of the mixtures at passage 3 and certainly at passage 6, showed a greater ratio of the virus carrying the transgene in the E1 parallel orientation compared with the E1 antiparallel version. By passage 10, the only viral species observed was the adenovector with the transgene in the E1 parallel orientation for both transgenes tested (hCMV(no intron)-FLgag-bGHpA and hCMV(no intron)-FLgag-SPA).

Analysis of the viral species from the plasmid mixing experiment #2 (Figure 8B) at passages 3 and 6 showed that the polyadenylation signals tested (bGHpA and SPA) did not have an effect on the growth of the virus. Even at passage 10 the two viral species in the mixture were still present in equal amounts.

5

10

15

20

25

30

EXAMPLE 11

Virus generation of an enhanced adenoviral construct - "MRK Ad5 HIV-1gag"

The results obtained from the competition study allowed us to make the following conclusions: (1) The packaging signal extension is beneficial; (2) Presence of E3 does enhance viral growth; (3) E1 parallel orientation is recommended; and (4) PolyA signals have no effect on the growth of the adenovirus.

MRK Ad5 HIV-1 gag exhibited the most desirable results. This construct contains the hCMV(no intron)-FLgag-bGHpA transgene inserted into the new E3+ adenovector backbone, MRKpAdHVE3, in the E1 parallel orientation. We have designated this adenovector MRK Ad5 HIV-1 gag. This construct was prepared as outlined below:

The pre-plasmid MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA was digested was Pac1 to release the vector backbone and 3.3 µg was transfected by calcium phosphate method (Amersham Pharmacia Biotech.) in a 6 cm dish containing PER.C6® cells at ~60% confluence. Once CPE was reached (7-10 days), the culture was freeze/thawed three times and the cell debris pelleted. 1 ml of this cell lysate was used to infect into a 6 cm dish containing PER.C6® cells at 80-90% confluence. Once CPE was reached, the culture was freeze/thawed three times and the cell debris pelleted. The cell lysate was then used to infect a 15 cm dish containing PER.C6[®] cells at 80-90% confluence. This infection procedure was continued and expanded at passage 6. The virus was then extracted from the cell pellet by CsCl method. Two bandings were performed (3-gradient CsCl followed by a continuous CsCl gradient). Following the second banding, the virus was dialyzed in A105 buffer. Viral DNA was extracted using pronase treatment followed by phenol chloroform. The viral DNA was then digested with *Hind*III and radioactively labeled with [33P]dATP. Following gel electrophoresis to separate the digestion products the gel was dried down on Whatman paper and then subjected to autoradiography. The digestion products were compared with the digestion products from the pre-plasmid (that had been digested with Pac1/HindIII prior to labeling). The expected sizes were observed, indicating that the virus had been successfully rescued. This strategy was used to rescue virus from each of the various adenovector plasmid constructs prepared.

30

5

10

15

20

25

EXAMPLE 12

Stability Analyses

5

10

15

20

25

30

35

To determine whether the various adenovector constructs (e.g., MRK Ad5 HIV-1 gag) show genetic stability, the viruses were each passaged continually. The viral DNA was analyzed at passages 3, 6 and 10. Each virus maintained its correct genetic structure. In addition, the stability of the MRK Ad5 HIV-1 gag was analyzed under propagation conditions similar to that performed in large scale production. For this analysis, the transfections of MRK Ad5 HIV-1 gag as well as three other adenoviral vectors were repeated and the virus was purified at P3. The three other adenovectors were as follows: (1) that comprising hCMV(no intron)-Flgag with a bGHpA terminator in an E3- adenovector backbone; (2) that comprising hCMV(no intron)-Flgag with a SPA termination signal in an E3+ adenovector backbone, and that comprising a mCMV-Flgag with a bGHpA terminator in an E3+ adenovector backbone. All of the vectors have the transgene inserted in the E1 parallel orientation. Viral DNA was analyzed by radioactive restriction analysis to confirm that it was correct before being delivered to fermentation cell culture for continued passaging in serum-free media. At P5 each of the four viruses were purified and the viral DNA extracted for analysis by the restriction digestion and radiolabeling procedure. This virus has subsequently been used in a series of studies (in vitro gag expression in COS cells, rodent study and rhesus monkey study) as will be described below. The viruses from P5 are shown in Figure 9.

The passaging under serum-free conditions was continued for the MRKHVE3 (transgene-less, obtained from MRKpAdHVE3 pre-plasmid) and the MRKAd5HIV-1gag (obtained from MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA pre-plasmid) viruses. Figure 10 shows viral DNA analysis by radioactive restriction digestion at passage 11 for MRKHVE3, MRKAd5HIV-1gagE3-, and passage 11 and 12 for MRKAd5HIV-1gag. Aside from the first lane which is the DNA marker lane, the next three lanes are virus from the pre-plasmid controls (controls based on the original virus) - MRKpAdHVE3 (also referred to as "pMRKHVE3"), MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA, and pMRKAd5gag(E3-), respectively. As seen in Figure 10, each of the viral DNA samples show the expected bands with no extraneous bands showing. This signifies that there are no major variant adenovirus species present that can be detected by autoradiography.

Figure 11 shows the results of viral competition study between MRKHVE3 and MRKAd5HIV-1gag. These viruses were mixed together at equal MOI (140 viral

particles each; 280 vp total) at passage 6 and continued to be passaged until P11. Aside from the first lane which is the DNA marker lane, the next two lanes are the pre-plasmid controls obtained from MRKpAdHVE3 and MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA. The next two lanes are the viral DNA from the starting viral material at passage six. The last two lanes are the competition studies performed in duplicate. The data in Figure 11 shows the effect the gag transgene in culture. Growth of a MRKAd5gag virus was compared with growth of a "transgene-less" MRKHVE3. These two viruses were infected at the same MOI (i.e. 140 vp each) at passage 6 and then passaged through to passage 11 and the viral pool was analyzed by radioactive restriction analysis. The data shows that one virus did not out compete the other. Therefore, the gag transgene did not show obvious signs of toxicity to the adenovirus.

10

15

20

25

30

Analysis by *Hind*III digestion shows that each virus specie is present in approximately equal amounts. As above, there does not appear to be signs of any extraneous bands. Figure 12 shows higher passage numbers for MRKAd5HIV-1gag grown under serum-containing conditions. The genome integrity again has been maintained and there is no evidence of rearrangements, even at the highest passage level (P21).

Each of the four vectors shown in Figure 9 were analyzed for amplification capacity. Table 4 below shows the QPA analysis used in the estimation of viral amplification ratios at P4. The determination of the amplification ratio for the original HIV-1 gag construct is based on the clinical lot at P12. It has been shown that amplification rates increases with higher passage number for the original virus. The reason for this observation is due to the emergence of variants which exhibit increased growth rates compared to the intact adenovector. With continued passaging of the original Ad gag vector, the level of variants increases and hence amplification rates increase also.

The MRK Ad5 HIV-1 gag virus has also been continually passaged under process conditions (i.e., serum-free media). Viral DNA extracted from passages 11 and 12 show no evidence of rearrangement.

Table 4:
Amplification Ratios Based on AEX and QPA Analysis of Virus Amplification from Passage 3 to Passage 4.

Ad gag construct	Amplification Ratio
MRKAd5gag	470
HCMV-Flgag-bGHpA [E3-]	115
HCMV-Flgag-SPA [E3+]	320
mCMV-FLgag-bGHpA [E3+]	420
Original construct *	40 - 50

5

EXAMPLE 13

10

Analytical Evaluation of the enhanced Ad5 Constructs

To study the effects of the transgene and the E3 gene on virus amplification, the enhanced adenoviral vector, MRK Ad5 HIV-1 gag, along with its transgene-less version (MRKpAdHVE3) and its E3- version (MRK Ad5 HIV-1 gag E3-), was studied for several passages under serum-free conditions. Table 5A shows the amplification ratios determined for passages P3 to P8 for MRK Ad5 HIV-1 gag. Within a certain MOI range, it has been determined that the virus output is directly proportional to the virus input. Therefore, the greater the number of virus particles per cell at infection, the greater the virus amount produced. Viral amplification ratios, on the other hand, are inversely proportional to the virus input. The lower the virus input, the greater the amplification ratio.

20

25

15

Table 5B shows the amplification rates of the new E3+ vector backbone MRKpAdHVE3. It has a significantly lower rate of amplification compared with the gag transgene containing version. This may be contributed to the larger size MRK Ad5 HIV-1 gag since it contains the transgene. This inclusion of the transgene brings the size of the adenovirus closer to the size of a wild type Ad5 virus. It is well known that adenoviruses amplify best when they are at close to their wild type genomic size.

^{*} This estimation is based on the clinical lot growth characteristics at Passage 12.

Wild type Ad5 is 35,935 bp. The MRKpAdHVE3 is 32, 905 bp in length. The enhanced adenovector MRK Ad5 HIV-1 gag is 35,453bp (See Figure 14 for vector map; see also Figure 15A-X show the complete pre-adenoviral vector sequence, which includes an additional 2,021 bp of the vector backbone).

5

10

Table 5C shows the amplification rates of the new E3- gag containing virus MRK Ad5 HIV-1 gag E3-. Once again, this virus shows lower growth rate than the enhanced adenoviral vector. This may be attributed to the decreased sized of this virus (due to the E3 gene deletion) compared with wild type Ad5. The MRK Ad5 HIV-1 gag E3- virus is 32,810 bp in length. This can be compared with the wild type Ad5 which is 35,935 bp and MRK Ad5 HIV-1 gag which is 35,453 bp in length.

Table 5A: Amplification ratios determined by AEX and QPA for MRKAd5gag over several continuous passaging in serum free media. Following P5, two replicate samples were taken (rep-1 and rep-2) and analyzed.

MRKAd5gag rep1

	Xv (10 ⁴ calls/tr Infection	il), Viability (%) Harvest	Harvest Time	Cell Passage Number	Titler 10" vp/ml culture	Ther 10° vp/cell	OPA 10° TCIO _M /mt	Ratio AEX:QPA	Amplification Rato	AEX Internal Control
P4	1,49, 81%	0.58, 50%	44	46	8.7	5.9	1.72	50	470 (MOI = 125)	
PS	1,38, 93%	0.68, 47%	48	49	6.7	4.9	1.38	49	170	
PB	1.04, 94%	0.68, 77%	47	48	5.8	5.6	1.42	41	200	
P7	1.50, 84%	0.98, 61%	49.5	50	3.9	1,4	0.87	40	50	
P7	1.09, 97%	0.76, 69%	50	52	5.2	4.7	1.70	81	170	
P8	1.03, 94%	0.88, 84%	47.5	54	9.0	8.7	1.10	812	210	
P9	0.89, 95%	0.89, 73%	47.5	56	4,4	4.9	1.03	43	175	3.12 2.84
P10	1.09, 91%	1.06, 66%	47.5	58	8.0	2.8	1.16	26	100	2.70 2.60
PII	1.19, 88%	0.88, 65%	47	60	3.6	3.0	1.15	31	110	2.70 2.70
P12	0,88, 91%	0.85, 63%	47.5	47	5.4	5.5	1.20	45	200	2.88 2.60
P13	1,00, 68%	0.70, 67%	49	49	5.8	5.8	1.11	52	210	3.18 3.18
P14	1,94, 92%	0.88, 67%	48	53	8.6	4.4	t		160	3.28 3.27
P15	0.97, 96%	0.64, 66%	47	47	6.9	7.1			250	3.12 2.01

Table 5B: Amplification ratios determined by AEX and QPA for MRKHVE3 over several continuous passaging in serum free media. MRKHVE3 is the new vector backbone which does NOT carry a transgene.

MRKHVE3

	Xv (10° ceils/i	ri), Viabliny (%)	Harvest Time	Cell Passage	Ytter	Titer	QPA	Ratio	Amplification	AEX
	Infection	Harvest	hpl	Number	10 ^{td} vp/ml culture	10° vp/cet	10° TCID _{eo} /ml	AEX:QPA	Ratio	Internal Control
P4	1.10, 97%	1.28, 79%	49	54	4.1	8.8	1.70	25	300 (MO) = 125)	
P5	0.82, 89%	1.18, 77%	47	. 48	4.3	4.7	1.24	35	170	
PB	1,55,88%	1.26, 76%	49.5	50	1.2	0.8	0.56	21	30	
P6	1.09, 97%	1.11,81%	49	52	4.0	3.6	1.16	84	130	
P7	1.17, 91%	1.22, 91%	47.5	54	3.7	3.2	0.50	74	110	
P8	0.98, 88%	1.41, 83%	48	56	2.1	2.1	0,47	45	75	3.12 2.84
P9	1.20, 89%	1.28, 81%	47.5	58	0.8	0.7	0.29	28	జ	2.70 2.60
P10	0.89, 82%	1.55, 86%	47	60	23	23	0.43	53	80	2.70 2.70
P11	1.07, 96%	1.25, 83%	48	47	2.7	2.5	0.41	66	90	2.86 2.80
P12	0.80, 91%	1.14, 80%	49,5	49	5.9	7.4	0.48	123	250	3.18 3.18
Pis	1.96, 95%	1.14, 85%	45.5	63	5.8	3.0			110	3.28 3.27
P14	0.97, 96%	1.03, 98%	46.5	47	9.4	8.7			350	3.12 2.91
P15	0.87, 99%	0.97, 59%	49.5	49	5.3	6.1	i		218	2.78 2.52

Table 5C. Amplification ratios determined by AEX and QPA for MRKAd5gag(E3-) over several continuous passaging in serum free media. This construct is identical to the MRKAd5gag construct except that this version is DELETED of the E3 gene.

5 .

MRKAd5gag(E3-)

	Xv (10 ⁴ cells/n infection	ri), Vieblity (%) Harvest	Harvest Time h.p.l.	Cell Passage Number	Titer 10 ^{co} vp/mi culture	Titer 10° vp/celi	QPA 10° TCID _{ED} Ami	Ratio AEX:QPA	Amplification Ratio	AEX Internal Control
P4	1.62, 77%	1.12, 62%	47.5	48	2.0	1.2	0.92	20	100 (MOI=125)	
P\$	1.16, 92%	0.62, 43%	49	49	3.3	2.9	0,99	34	100	
P6	1.71, 86%	0.20, 10%	49	50	4.7	2.7	1.70	_ 28	100	
P6	1.09, 97%	0.63, 54%	49.5	52	5.4	5.0	1.76	31	180	
P7	1,17, 91%	0.98, 72%	47.50	54	7.1	6.1	0,67	108	220	
PB	0.98, 88%	0.77, 48%	48	58	3.1	3.2	0.66	47	115	3.12 2.84
P9	1.20, 89%	1,03,72%	48	58	1.8	1.5	0.67	32	55	2.70 2.60
P10	0.99, 82%	0.80, 62%	46.5	60	3.2	3.2	83.0	47	115	2.70 2.70
P11	1.07, 96%	0.88, 70%	48.5	47	5.9	5.5	0.68	87	200	2.88 2.60
P12	0.80, 81%	0.67, 59%	50	49	5.1	6.4	0.72	71	230	3.18 3.18
P13	1.96, 95%	0.91, 59%	45.5	53	7.4	3.8			135	3.28 3.27
P14	0.97, 96%	0.81, 74%	48	47	6.8	7.0			250	3.12 2.91
P18	0.87, 99%	0.84, 58%	49	49	4,8	5.5			196	2.78 2.52

EXAMPLE 14

Gag Expression Analysis of the Novel Constructs

In vitro gag analysis of the MRK Ad5 HIV-1 gag and the original HIV-gag vectors (research and clinical lot) show comparable gag expression. The clinical lot shows only a slightly reduced gag expression level. The most noticeable difference is with the mCMV vector. This vector shows roughly 3 fold lower expression levels compared with the other vectors tested (which all contain hCMV promoters). The mCMV-FLgag with bGHpA assay was performed three times using different propagation and purification lots and it consistently exhibited weaker gag expression.

EXAMPLE 15

Evaluation of MRK Ad5 HIV-1 gag and Other gag-Containing Adenovectors in Balb/c Mice

Cohorts of 10 balb/c mice were vaccinated intramuscularly with escalating doses of MRK Ad5 HIV-1 gag, and the research and clinical lots of original Ad5HIV-1gag. Serum samples were collected 3 weeks post dose 1 and analyzed by anti-p24 sandwich ELISA.

Anti-p24 titers in mice that received MRK Ad5 HIV-1 gag (10⁷ and 10⁹ vp(viral particle) doses) were comparable (Figure 13) to those of the research lot of Ad5HIV-1 gag, for which much of the early rhesus data were generated on. These titers were also comparable when E3 is deleted (MRKAd5hCMVgagbGHpA(E3-)) or SPA is substituted for bGHpA terminator (MRKAd5 hCMV-gag-SPA (E3+)) or murine CMV promoter is used in place of hCMV (MRKAd5 mCMV-gag-bGHpA (E3+)) in the MRKAd5 backbone.

The results shown in Table 7 indicate that the three other vectors (in addition to the preferred vector, MRK Ad5 HIV-1 gag, are also capable of inducing strong anti-gag antibody responses in mice. Interestingly enough, while the mCMV-FLgag construct containing bGHpA and E3+ in an E1 parallel orientation showed lowest gag expression in the COS cell *in vitro* infection (Table 6) in comparison with the other vectors tested, it generated the greatest anti-gag antibody response this *in vivo* Balb/c study. Table 7 also shows a dose response in anti-gag antibody production in both the research and the clinical lot. As expected, the clinical lot shows reduced anti-gag antibody induction at each dosage level compared to the same dosage used for the research lot.

Table 6: In vitro analysis for gag expression in COS cells by Elisa assay.

20

25

15

5

10

Viral Vectors ^a	µg gag/4.8x10e5 COS/10e8 parts/48hr
MRKAd5gag ^b	1.40
Clinical lot Ad5gag ^c	1.28
Research lot Ad5gag ^d	1.32
MCMVFL-gagbGHpA ^c	0.42

^a A_{260nm} absorbance readings taken for viral particle determinations.

b MRKAd5gag was produced in serum free conditions and purified at P5.

^c Clinical lot# Ad5gagFN0001

d Research Ad5FLgag lot# 6399

[°] mCMVFL-gagbGHpA was produced in serum free conditions and purified at P5.

Table 7: mHIV020 Anti-p24 Ab Titers in Balb/c mice (n=10) vaccinated with various Adgag constructs and lots (3 week post dose1).

Group	Vaccine	Dose (vp)	GMT	SE upper	SE lower
1	*MRKAd5gag	10^7	25600	5877	4780
2	a a	10^9	409600	94028	76473
l					
3	hCMV FL-gag bGHpA [E3-] →	10^7	7352	2077	1620
4		10^9	235253	59767	47659
5	hCMV FL-gag SPA [E3+] →	10^7	12800	9905	236
6	HOWAY E-gay or A [Lot] ->	10^9	310419	99181	75165
•		10.3	010413	33731	70.00
7	^b mCMV FL-gag bGHpA [E3+] →	10^7	44572	23504	15389
8	and the general section of the secti	10/9	941014	239068	190636
					1
9	^c hCMV FL-gag bGHpA [E3-] ←	10^7	3676	934	745
10	•	10^9	117627	17491	15227
	research lot hCMV intronA FL-gag bGHpA [E3-] <-	10^6	528	262	175
11 12	Lessarch for tionia introtty Le-daß pariby [eq-]	10^7	14703	5274	3882
13		10/8	58813	14942	11915
14		10/9	204800	53232	42250
'*		.03	20,000	1 3325	1 12.00
15	clinical lot hCMVintronA FL-gag bGHpA [E3-] <-	10^6	230	82	61
16	1	10^7	4222	3405	1138
17	•	10^8	19401	3939	3274
18	н	10^9	89144	25187	19639
				١ ـ	_
l 19	Naïve	none	93	7	6

*2x50 µL I.m. (quad) injections/animal

P.I.s: Youil, Chen, Casimiro Vaccination: T. Toner, Q. Su

Assay: M. Chen

5

10

EXAMPLE 16

Comparison of Humoral and Cellular Responses Towards the Original Ad-gag Construct with the New MRK Ad5 HIV-1 gag in Rhesus Monkeys

Cohorts of 3 rhesus monkeys were vaccinated intramuscularly with MRK Ad5 HIV-1 gag or the clinical Ad5gag bulk at two doses, 10^{11} vp and 10^9 vp. Immunizations were conducted at week 0, 4, and 25. Serum and PBMC samples were collected at selected time points. The serum sample were assayed for anti-p24 Ab titers (using competitive based assay) and the PBMCs for antigen-specific IFN-gamma secretion following overnight stimulation with gag 20-mer peptide pool (via ELISpot assay).

The results shown in Table 8 indicate comparable responses with respect to the generation of anti-gag antibodies. The frequencies of gag-specific T cells in

^aThe structure of MRKAd5gag is: hCMVFL-gagbGHpA [E3+] \rightarrow The <u>same lot</u> of MRKAd5gag used in this rodent study was used in the Rhesus monkey study (Tables 7 and 8).

^bThe same lot of mCMVFL-gagbGHpA[E3+] used in the in vitro study (Table 6) ws used here.

This construct was designed by Volker Sandig. It contains a shorter version of the hCMV promoter than that used in the MRK constructs. The adenovector backbone is identical to the original backbone used in the original Adgag vector. Expression at 10e7 dose from this vector is 7 fold lower then the same dose of the MRKAd5gag and 4 fold lower than the research lot.

peripheral blood assummarized in Table 9 demonstrate a strong cellular immune response generated after a single dose with the new construct MRK Ad5 HIV-1 gag. The responses are also boostable with second dose of the same vector. The vector is also able to induce CD8+ T cell responses (as evident by remaining spot counts after CD4+ depletion of PBMCs) which are responsible for cytotoxic activity.

Table 8 Anti-p24 antibody titers (in mMU/mL) in rhesus macaques immunized with

gag-expressing adenovectors (Protocol HIV203).

Vaccine	Pre	Wk4	Wk8	Wk 12	Wk 16	Wk 20	Wk 25	Wk 28
MR KAd5gaga, 10^11 vp								
97N010	<10	118	5528	11523	7062	21997	ND	51593
97N1 16	<10	62	772	1447	1562	2174	ND	20029
98X007	<10	66.	3353	6156	6845	3719	ND	24031
MR K Ad5gag, 10^9 vp								
97N120	<10	51	204	318	366	482	ND	6550
97N144	<10	18	118	274	706	888	ND	7136
98X008	<10	15	444	386	996	1072	ND	12851
Ad5gag ^b , Clinical Lot, 10^11 vp								
97X001	<10_	87	2579	4718	7174	7250	ND	69226
97N146	<10	72	3604	7380	7526	18906	ND	60283
98X009	<10	78	4183	3946	3124	6956	ND	26226
Ad5gag, Clinical Lat, 10^9 vp		<u> </u>	<u> </u>				<u></u>	
97N020	<10	<10	143	371	390	1821	ND	17177
97X003	<10	<10	39	93	156	596	ND	2053
98X012	<10	81	342	717	956	1558	ND	11861
MRKAd5gag (hCMV, bGHpA, E3+)								
bariginal Actigag vector (hCMV/Intra	n A bGHp	A, E3-), lott	FN0001					
ND, not determined	<u></u>	<u> </u>		<u> </u>	<u> </u>	L		

5

Table 9. Number of gag-specific T cells per million peripheral blood mononuclear cells (PBMCs) in rhesus monkeys immunized with gag-expressing adenovectors. Also included are those frequencies in PBMCs depleted of CD4⁺ T cells.

Grp #	Vaccination	Monkey ID		Wk		Wk		Wk	Iel			Wk		Wk
	7 ≃0,4,25 wig		Media	Gog H ^b	Media	Gog H	Medio	Goog H	Media	Gog H	Media	Gog H	Media	Gog H
1	MRKAdāgag 10/11 vp	97ND10 97ND10(CD4-)	6 4	89 38	0	395	0 3	1058 993	0	1174	3 0	775 76	4	1074 594
		97N116	1 1	396 676	1	609	0	534 593	4	395	1	261 184	0	408 666
		97N116(CD4-) 98X007	11	579	0	1304	3	2193	1	2118	3	1588	Ō	2113
		98X007(CD4-)	20	965			0	2675			٥	1656	٥	1278
2	MRKAd5gag	97N120	5	275	1	249	4	141	4	119	S	206 75	4	219 219
	10/9 vp	97N120(CD4-) 97N144	11	170 236	6	438	0 1	85 318	3	256	i	98	5	373
		97N144(CD4-) 98X008	6	148 988	١,	1090	0	285 891	₄	673	ND 3	NO 473	0 5	625 735
		98X008(CD4-)	14	696	'		ŏ	1175		""	ō	391	4	848
3	AdSgoog dinked left	97X001	0	261	1	485	0	817	0	1220b	1	894	0	1858
	10 ⁴ 11 vp	97X001(CD4-) 97N146	10	283 150	1	465	3	996 339	1	1272	3	1010 1238	0 3	1123 1785
		97N146(CD4-)	6	133	۱ a	339	0	370 559		896	0	654 384		971 1748
		98X009(CD4-)	0	73	,	333	ő	333	١		ó	225	ŏ	644
4	AdSpoordinate lat	97N020 97N020(CD4-)	3	30 29	1	101	0	66 15	0	36	0	26 1	0	41 16
	1074	97X003	4	68	5	134	ò	18	יו	38	4	38	ô	81
		97X003(CD4-) 98X012 98X012(CD4-)	9 5 11	95 70	3	54	1 0	6 34 11	0	18	00	20 8		121 41
5	Noive	96RD41 053F	B 14	8 16	1 5	16,	0 20	D 14	19	0 15	0 10	0 15	24	0

Bosed on either 4x10/6 or 2x10/6 calls per well (depending on spot density)

ND, not determined

5

10

15

20

The adenovectors described herein and, particularly, MRK Ad5 HIV-1 gag, represent very promising HIV-gag adenovectors with respect to their enhanced growth characteristics in both serum and, more importantly, in serum-free media conditions. In comparison with the current HIV-1 gag adenovector construct, MRK Ad5 HIV-1 gag shows a 5-10 fold increased amplification rate. We have shown that it is genetically stable at passage 21. This construct is able to generate significant cellular immune responses in vivo even at a relatively low dose of 10^9 vp. The potency of the MRKAd5gag construct is comparable to, if not better than the original HIV-1gag vector as shown in this rhesus monkey study.

EXAMPLE 17 CODON OPTIMIZED HIV-1 POL AND CODON OPTIMIZED HIV-1 POL MODIFICATIONS

The open reading frames for the various synthetic *pol* genes disclosed herein comprise coding sequences for the reverse transcriptase (or RT which consists of a polymerase and RNase H activity) and integrase (IN). The protein sequence is based

[&]quot;mode or no pecifide control

"Pool of 20-capeptides overlapping by 10 caland encompassing the popsequence

on that of Hxb2r, a clonal isolate of IIIB; this sequence has been shown to be closest to the consensus clade B sequence with only 16 nonidentical residues out of 848 (Korber, et al., 1998, Human retroviruses and AIDS, Los Alamos National Laboratory, Los Alamos, New Mexico). The skilled artisan will understand after review of this specification that any available HIV-1 or HIV-2 strain provides a potential template for the generation of HIV pol DNA vaccine constructs disclosed herein. It is further noted that the protease gene is excluded from the DNA vaccine constructs of the present invention to insure safety from any residual protease activity in spite of mutational inactivation. The design of the gene sequences for both wildtype (wt-pol) and inactivated pol (IA-pol) incorporates the use of human preferred ("humanized") codons for each amino acid residue in the sequence in order to maximize in vivo mammalian expression (Lathe, 1985, J. Mol. Biol. 183:1-12). As can be discerned by inspecting the codon usage in SEQ ID NOs: 1, 3, 5 and 7, the following codon usage for mammalian optimization is preferred: Met (ATG), Gly (GGC), Lys (AAG), Trp (TGG), Ser (TCC), Arg (AGG), Val (GTG), Pro (CCC), Thr (ACC), Glu (GAG); Leu (CTG), His (CAC), Ile (ATC), Asn (AAC), Cys (TGC), Ala (GCC), Gln (CAG), Phe (TTC) and Tyr (TAC). For an additional discussion relating to mammalian (human) codon optimization, see WO 97/31115 (PCT/US97/02294). which, as noted elsewhere in this specification, is hereby incorporated by reference. It is intended that the skilled artisan may use alternative versions of codon optimization or may omit this step when generating HIV pol vaccine constructs within the scope of the present invention. Therefore, the present invention also relates to non-codon optimized versions of DNA molecules and associated recombinant adenoviral HIV vaccines which encode the various wild type and modified forms of the HIV Pol protein disclosed herein. However, codon optimization of these constructs is a preferred embodiment of this invention.

10

15

20

25

30

35

A particular embodiment of this portion of the invention comprisies codon optimized nucleotide sequences which encode wt-pol DNA constructs (herein, "wt-pol" or "wt-pol (codon optimized))" wherein DNA sequences encoding the protease (PR) activity are deleted, leaving codon optimized "wild type" sequences which encode RT (reverse transcriptase and RNase H activity) and IN integrase activity. A DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:1, the open reading frame being contained from an initiating Met residue at nucleotides 10-12 to a termination codon from nucleotides 2560-2562. SEQ ID NO:1 is as follows:

AGATCTACCA TGGCCCCCAT CTCCCCCATT GAGACTGTGC CTGTGAAGCT GAAGCCTGGC

ATGGATGGCC CCAAGGTGAA GCAGTGGCCC CTGACTGAGG AGAAGATCAA GGCCCTGGTG

	GAAATCTGCA	CTGAGATGGA	GAAGGAGGC	AAAATCTCCA	AGATTGGCCC	CGAGAACCCC
	TACAACACCC	CTGTGTTTGC	CATCAAGAAG	AAGGACTCCA	CCAAGTGGAG	GAAGCTGGTG
	GACTTCAGGG	AGCTGAACAA	GAGGACCCAG	GACTTCTGGG	AGGTGCAGCT	GGGCATCCCC
	CACCCCGCTG	GCCTGAAGAA	GAAGAAGTCT	GTGACTGTGC	TGGATGTGGG	GGATGCCTAC
5	TTCTCTGTGC	CCCTGGATGA	GGACTTCAGG	AAGTACACTG	CCTTCACCAT	CCCCTCCATC
	AACAATGAGA	CCCCTGGCAT	CAGGTACCAG	TACAATGTGC	TGCCCCAGGG	CTGGAAGGGC
	TCCCCTGCCA	TCTTCCAGTC	CTCCATGACC	AAGATCCTGG	AGCCCTTCAG	GAAGCAGAAC
	CCTGACATTG	TGATCTACCA	GTACATGGAT	GACCTGTATG	TGGGCTCTGA	CCTGGAGATT
	GGGCAGCACA	GGACCAAGAT	TGAGGAGCTG	AGGCAGCACC	TGCTGAGGTG	GGGCCTGACC
10	ACCCCTGACA	AGAAGCACCA	GAAGGAGCCC	CCCTTCCTGT	GGATGGGCTA	TGAGCTGCAC
	CCCGACAAGT	GGACTGTGCA	GCCCATTGTG	CTGCCTGAGA	AGGACTCCTG	GACTGTGAAT
	GACATCCAGA	AGCTGGTGGG	CAAGCTGAAC	TGGGCCTCCC	AAATCTACCC	TGGCATCAAG
	GTGAGGCAGC	TGTGCAAGCT	GCTGAGGGGC	ACCAAGGCCC	TGACTGAGGT	GATCCCCCTG
	ACTGAGGAGG	CTGAGCTGGA	GCTGGCTGAG	AACAGGGAGA	TCCTGAAGGA	GCCTGTGCAT
15	GGGGTGTACT	ATGACCCCTC	CAAGGACCTG	ATTGCTGAGA	TCCAGAAGCA	GGGCCAGGGC
	CAGTGGACCT	ACCAAATCTA	CCAGGAGCCC	TTCAAGAACC	TGAAGACTGG	CAAGTATGCC
	AGGATGAGGG	GGGCCCACAC	CAATGATGTG	AAGCAGCTGA	CTGAGGCTGT	GCAGAAGATC
	ACCACTGAGT	CCATTGTGAT	CTGGGGCAAG	ACCCCCAAGT	TCAAGCTGCC	CATCCAGAAG
	GAGACCTGGG	AGACCTGGTG	GACTGAGTAC	TGGCAGGCCA	CCTGGATCCC	TGAGTGGGAG
20	TTTGTGAACA	CCCCCCCCT	GGTGAAGCTG	TGGTACCAGC	TGGAGAAGGA	GCCCATTGTG
	GGGGCTGAGA	CCTTCTATGT	GGATGGGGCT	GCCAACAGGG	AGACCAAGCT	GGGCAAGGCT
	GGCTATGTGA	CCAACAGGGG	CAGGCAGAAG	GTGGTGACCC	TGACTGACAC	CACCAACCAG
	AAGACTGAGC	TCCAGGCCAT	CTACCTGGCC	CTCCAGGACT	CTGGCCTGGA	GGTGAACATT
	GTGACTGACT	CCCAGTATGC	CCTGGGCATC	ATCCAGGCCC	AGCCTGATCA	GTCTGAGTCT
25	GAGCTGGTGA	ACCAGATCAT	TGAGCAGCTG	ATCAAGAAGG	AGAAGGTGTA	CCTGGCCTGG
	GTGCCTGCCC	ACAAGGGCAT	TGGGGGCAAT	GAGCAGGTGG	ACAAGCTGGT	GTCTGCTGGC
	ATCAGGAAGG	TGCTGTTCCT	GGATGGCATT	GACAAGGCCC	AGGATGAGCA	TGAGAAGTAC
	CACTCCAACT	GGAGGGCTAT	GGCCTCTGAC	TTCAACCTGC	CCCCTGTGGT	GGCTAAGGAG
	ATTGTGGCCT	CCTGTGACAA	GTGCCAGCTG	AAGGGGGAGG	CCATGCATGG	GCAGGTGGAC
30	TGCTCCCCTG	GCATCTGGCA	GCTGGACTGC	ACCCACCTGG	AGGGCAAGGT	GATCCTGGTG
	GCTGTGCATG	TGGCCTCCGG	CTACATTGAG	GCTGAGGTGA	TCCCTGCTGA	GACAGGCCAG
	GAGACTGCCT	ACTTCCTGCT	GAAGCTGGCT	GGCAGGTGGC	CTGTGAAGAC	CATCCACACT
	GACAATGGCT	CCAACTTCAC	TGGGGCCACA	GTGAGGGCTG	CCTGCTGGTG	GGCTGGCATC
	AAGCAGGAGT	TTGGCATCCC	CTACAACCCC	CAGTCCCAGG	GGGTGGTGGA	GTCCATGAAC
35	· -					GAAGACAGCT
	GTGCAGATGG	CTGTGTTCAT	CCACAACTTC	AAGAGGAAGG	GGGGCATCGG	GGGCTACTCC

GCTGGGGAGA GGATTGTGGA CATCATTGCC ACAGACATCC AGACCAAGGA GCTCCAGAAG

CAGATCACCA AGATCCAGAA CTTCAGGGTG TACTACAGGG ACTCCAGGAA CCCCCTGTGG

AAGGGCCCTG CCAAGCTGCT GTGGAAGGGG GAGGGGGCTG TGGTGATCCA GGACAACTCT

GACATCAAGG TGGTGCCCAG GAGGAAGGCC AAGATCATCA GGGACTATGG CAAGCAGATG

GCTGGGGATG ACTGTGTGGC CTCCAGGCAG GATGAGGACT AAAGCCCGGG CAGATCT (SEQ

ID NO:1).

The open reading frame of the wild type pol construct disclosed as SEQ ID NO:1 contains 850 amino acids, disclosed herein as SEQ ID NO:2, as follows: Met Ala Pro Ile Ser Pro Ile Glu Thr Val Pro Val Lys Leu Lys Pro Gly Met Asp Gly Pro Lys Val Lys Gln Trp Pro Leu Thr Glu Glu Lys 10 Ile Lys Ala Leu Val Glu Ile Cys Thr Glu Met Glu Lys Glu Gly Lys Ile Ser Lys Ile Gly Pro Glu Asn Pro Tyr Asn Thr Pro Val Phe Ala Ile Lys Lys Lys Asp Ser Thr Lys Trp Arg Lys Leu Val Asp Phe Arg Glu Leu Asn Lys Arg Thr Gln Asp Phe Trp Glu Val Gln Leu Gly Ile pro His Pro Ala Gly Leu Lys Lys Lys Ser Val Thr Val Leu Asp Val Gly Asp Ala Tyr Phe Ser Val Pro Leu Asp Glu Asp Phe Arg Lys Tyr Thr Ala Phe Thr Ile Pro Ser Ile Asn Asn Glu Thr Pro Gly Ile Arg Tyr Gln Tyr Asn Val Leu Pro Gln Gly Trp Lys Gly Ser Pro Ala Ile Phe Gln Ser Ser Met Thr Lys Ile Leu Glu Pro Phe Arg Lys Gln 20 Asn Pro Asp Ile Val Ile Tyr Gln Tyr Met Asp Asp Leu Tyr Val Gly Ser Asp Leu Glu Ile Gly Gln His Arg Thr Lys Ile Glu Glu Leu Arg Gln His Leu Leu Arg Trp Gly Leu Thr Thr Pro Asp Lys Lys His Gln Lys Glu Pro Pro Phe Leu Trp Met Gly Tyr Glu Leu His Pro Asp Lys Trp Thr Val Gln Pro Ile Val Leu Pro Glu Lys Asp Ser Trp Thr Val Asn Asp Ile Gln Lys Leu Val Gly Lys Leu Asn Trp Ala Ser Gln Ile 25 Tyr Pro Gly Ile Lys Val Arg Gln Leu Cys Lys Leu Arg Gly Thr Lvs Ala Leu Thr Glu Val Ile Pro Leu Thr Glu Glu Ala Glu Leu Glu Leu Ala Glu Asn Arg Glu Ile Leu Lys Glu Pro Val His Gly Val Tyr Tyr Asp Pro Ser Lys Asp Leu Ile Ala Glu Ile Gln Lys Gln Gly Gln Gly Gln Trp Thr Tyr Gln Ile Tyr Gln Glu Pro Phe Lys Asn Leu Lys 30 Thr Gly Lys Tyr Ala Arg Met Arg Gly Ala His Thr Asn Asp Val Lys Gln Leu Thr Glu Ala Val Gln Lys Ile Thr Thr Glu Ser Ile Val Ile Trp Gly Lys Thr Pro Lys Phe Lys Leu Pro Ile Gln Lys Glu Thr Trp Glu Thr Trp Trp Thr Glu Tyr Trp Gln Ala Thr Trp Ile Pro Glu Trp Glu Phe Val Asn Thr Pro Pro Leu Val Lys Leu Trp Tyr Gln Leu Glu Lys Glu Pro Ile Val Gly Ala Glu Thr Phe Tyr Val Asp Gly Ala Ala

Asn Arg Glu Thr Lys Leu Gly Lys Ala Gly Tyr Val Thr Asn Arg Gly Arg Gln Lys Val Val Thr Leu Thr Asp Thr Thr Asn Gln Lys Thr Glu Leu Gln Ala Ile Tyr Leu Ala Leu Gln Asp Ser Gly Leu Glu Val Asn Ile Val Thr Asp Ser Gln Tyr Ala Leu Gly Ile Ile Gln Ala Gln Pro Asp Gln Ser Glu Ser Glu Leu Val Asn Gln Ile Ile Glu Gln Leu Ile Lys Lys Glu Lys Val Tyr Leu Ala Trp Val Pro Ala His Lys Gly Ile Gly Gly Asn Glu Gln Val Asp Lys Leu Val Ser Ala Gly Ile Arg Lys Val Leu Phe Leu Asp Gly Ile Asp Lys Ala Gln Asp Glu His Glu Lys Tyr His Ser Asn Trp Arg Ala Met Ala Ser Asp Phe Asn Leu Pro Pro Val Val Ala Lys Glu Ile Val Ala Ser Cys Asp Lys Cys Gln Leu Lys 10 Gly Glu Ala Met His Gly Gln Val Asp Cys Ser Pro Gly Ile Trp Gln Leu Asp Cys Thr His Leu Glu Gly Lys Val Ile Leu Val Ala Val His Val Ala Ser Gly Tyr Ile Glu Ala Glu Val Ile Pro Ala Glu Thr Gly Gln Glu Thr Ala Tyr Phe Leu Leu Lys Leu Ala Gly Arg Trp Pro Val Lys Thr Ile His Thr Asp Asn Gly Ser Asn Phe Thr Gly Ala Thr Val 15 Arg Ala Ala Cys Trp Trp Ala Gly Ile Lys Gln Glu Phe Gly Ile Pro Tyr Asn Pro Gln Ser Gln Gly Val Val Glu Ser Met Asn Lys Glu Leu Lys Lys Ile Ile Gly Gln Val Arg Asp Gln Ala Glu His Leu Lys Thr Ala Val Gln Met Ala Val Phe Ile His Asn Phe Lys Arg Lys Gly Gly Ile Gly Gly Tyr Ser Ala Gly Glu Arg Ile Val Asp Ile Ile Ala Thr 20 Asp Ile Gln Thr Lys Glu Leu Gln Lys Gln Ile Thr Lys Ile Gln Asn Phe Arg Val Tyr Tyr Arg Asp Ser Arg Asn Pro Leu Trp Lys Gly Pro Ala Lys Leu Leu Trp Lys Gly Glu Gly Ala Val Val Ile Gln Asp Asn Ser Asp Ile Lys Val Val Pro Arg Lys Ala Lys Ile Ile Arg Asp TVT Gly Lys Gln Met Ala Gly Asp Asp Cys Val Ala Ser Arg Gln Asp 25 Glu Asp (SEQ ID NO:2).

The present invention especially relates to an adenoviral vector vaccine which comprises a codon optimized HIV-1 DNA pol construct wherein, in addition to deletion of the portion of the wild type sequence encoding the protease activity, a combination of active site residue mutations are introduced which are deleterious to HIV-1 pol (RT-RH-IN) activity of the expressed protein. Therefore, the present invention preferably relates to an adenoviral HIV-1 DNA pol-based vaccine wherein the construct is devoid of DNA sequences encoding any PR activity, as well as containing a mutation(s) which at least partially, and preferably substantially, abolishes RT, RNase and/or IN activity. One type of HIV-1 pol mutant which is part and parcel of an adenoviral vector vaccine may include but is not limited to a mutated

30

35

DNA molecule comprising at least one nucleotide substitution which results in a point mutation which effectively alters an active site within the RT, RNase and/or IN regions of the expressed protein, resulting in at least substantially decreased enzymatic activity for the RT, RNase H and/or IN functions of HIV-1 Pol. In a preferred embodiment of this portion of the invention, a HIV-1 DNA pol construct contains a mutation or mutations within the Pol coding region which effectively abolishes RT, RNase H and IN activity. An especially preferable HIV-1 DNA pol construct in a DNA molecule which contains at least one point mutation which alters the active site of the RT, RNase H and IN domains of Pol, such that each activity is at least substantially abolished. Such a HIV-1 Pol mutant will most likely comprise at least one point mutation in or around each catalytic domain responsible for RT, RNase H and IN activity, respectfully. To this end, an especially preferred HIV-1 DNA pol construct is exemplified herein and contains nine codon substitution mutations which results in an inactivated Pol protein (IA Pol: SEQ ID NO:4, Figure 17A-C) which has no PR, RT, RNase or IN activity, wherein three such point mutations reside within each of the RT, RNase and IN catalytic domains. Therefore, an especially preferred exemplification is an adenoviral vaccine which comprises, in an appropriate fashion, a DNA molecule which encodes IA-pol, which contains all nine mutations as shown below in Table 1. An additional preferred amino acid residue for substitution is Asp551, localized within the RNase domain of Pol. Any combination of the mutations disclosed herein may suitable and therefore may be utilized as an IA-Pol-based vaccine of the present invention. While addition and deletion mutations are contemplated and within the scope of the invention, the preferred mutation is a point mutation resulting in a substitution of the wild type amino acid with an alternative amino acid residue.

5

10

15

20

25

T	•	L	1	_	1
1	и	b	1	┖	

	wt aa	aa residue	mutant aa	enzyme function
	Asp	112	Ala	RT
	Asp	187	Ala	RT
30	Asp	188	Ala	RT
	Asp .	445	. Ala	RNase H
	Glu	480	Ala	RNase H
	Asp	500	Ala	RNase H
	Asp	626	Ala	IN
35	Asp	678	Ala	IN
	Glu	714	Ala	IN

It is preferred that point mutations be incorporated into the IApol mutant adenoviral vaccines of the present invention so as to lessen the possibility of altering epitopes in and around the active site(s) of HIV-1 Pol.

To this end, SEQ ID NO:3 discloses the nucleotide sequence which codes for a codon optimized pol in addition to the nine mutations shown in Table 1, disclosed as follows, and referred to herein as "IApol":

5

10

15

20

25

30

35

AGATCTACCA TGGCCCCCAT CTCCCCCATT GAGACTGTGC CTGTGAAGCT GAAGCCTGGC ATGGATGGCC CCAAGGTGAA GCAGTGGCCC CTGACTGAGG AGAAGATCAA GGCCCTGGTG GAAATCTGCA CTGAGATGGA GAAGGAGGGC AAAATCTCCA AGATTGGCCC CGAGAACCCC TACAACACCC CTGTGTTTGC CATCAAGAAG AAGGACTCCA CCAAGTGGAG GAAGCTGGTG GACTTCAGGG AGCTGAACAA GAGGACCCAG GACTTCTGGG AGGTGCAGCT GGGCATCCCC CACCCCGCTG GCCTGAAGAA GAAGAAGTCT GTGACTGTGC TGGCTGTGGG GGATGCCTAC TTCTCTGTGC CCCTGGATGA GGACTTCAGG AAGTACACTG CCTTCACCAT CCCCTCCATC AACAATGAGA CCCCTGGCAT CAGGTACCAG TACAATGTGC TGCCCCAGGG CTGGAAGGGC TCCCCTGCCA TCTTCCAGTC CTCCATGACC AAGATCCTGG AGCCCTTCAG GAAGCAGAAC CCTGACATTG TGATCTACCA GTACATGGCT GCCCTGTATG TGGGCTCTGA CCTGGAGATT GGGCAGCACA GGACCAAGAT TGAGGAGCTG AGGCAGCACC TGCTGAGGTG GGGCCTGACC ACCCCTGACA AGAAGCACCA GAAGGAGCCC CCCTTCCTGT GGATGGGCTA TGAGCTGCAC CCCGACAAGT GGACTGTGCA GCCCATTGTG CTGCCTGAGA AGGACTCCTG GACTGTGAAT GACATCCAGA AGCTGGTGGG CAAGCTGAAC TGGGCCTCCC AAATCTACCC TGGCATCAAG GTGAGGCAGC TGTGCAAGCT GCTGAGGGGC ACCAAGGCCC TGACTGAGGT GATCCCCCTG ACTGAGGAGG CTGAGCTGGA GCTGGCTGAG AACAGGGAGA TCCTGAAGGA GCCTGTGCAT GGGGTGTACT ATGACCCCTC CAAGGACCTG ATTGCTGAGA TCCAGAAGCA GGGCCAGGGC CAGTGGACCT ACCAAATCTA CCAGGAGCCC TTCAAGAACC TGAAGACTGG CAAGTATGCC AGGATGAGGG GGGCCCACAC CAATGATGTG AAGCAGCTGA CTGAGGCTGT GCAGAAGATC ACCACTGAGT CCATTGTGAT CTGGGGCAAG ACCCCCAAGT TCAAGCTGCC CATCCAGAAG GAGACCTGGG AGACCTGGTG GACTGAGTAC TGGCAGGCCA CCTGGATCCC TGAGTGGGAG TTTGTGAACA CCCCCCCCT GGTGAAGCTG TGGTACCAGC TGGAGAAGGA GCCCATTGTG GGGGCTGAGA CCTTCTATGT GGCTGGGGCT GCCAACAGGG AGACCAAGCT GGGCAAGGCT GGCTATGTGA CCAACAGGGG CAGGCAGAAG GTGGTGACCC TGACTGACAC CACCAACCAG AAGACTGCCC TCCAGGCCAT CTACCTGGCC CTCCAGGACT CTGGCCTGGA GGTGAACATT GTGACTGCCT CCCAGTATGC CCTGGGCATC ATCCAGGCCC AGCCTGATCA GTCTGAGTCT GTGCCTGCCC ACAAGGGCAT TGGGGGCAAT GAGCAGGTGG ACAAGCTGGT GTCTGCTGGC ATCAGGAAGG TGCTGTTCCT GGATGGCATT GACAAGGCCC AGGATGAGCA TGAGAAGTAC CACTCCAACT GGAGGGCTAT GGCCTCTGAC TTCAACCTGC CCCCTGTGGT GGCTAAGGAG

ATTGTGGCCT CCTGTGACAA GTGCCAGCTG AAGGGGAGG CCATGCATGG GCAGGTGGAC
TGCTCCCTG GCATCTGGCA GCTGGCCTGC ACCCACCTGG AGGGCAAGGT GATCCTGGTG
GCTGTGCATG TGGCCTCCGG CTACATTGAG GCTGAGGTGA TCCCTGCTGA GACAGGCCAG
GAGACTGCCT ACTTCCTGCT GAAGCTGGCT GGCAGGTGGC CTGTGAAGAC CATCCACACT
GCCAATGGCT CCAACTTCAC TGGGGCCACA GTGAGGGCTG CCTGCTGGTG GGCTGGCATC
AAGCAGGAGT TTGGCATCCC CTACAACCCC CAGTCCCAGG GGGTGGTGGC CTCCATGAAC
AAGGAGCTGA AGAAGATCAT TGGGCAGGTG AGGGACCAGG CTGAGCACCT GAAGACAGCT
GTGCAGATGG CTGTGTTCAT CCACAACTTC AAGAGGAAGG GGGCCATCGG GGGCTACTCC
GCTGGGGAGA GGATTGTGGA CATCATTGCC ACAGACATCC AGACCAACGGA GCTCCAGAAG
CAGATCACCA AGATCCAGAA CTTCAGGGTG TACTACAGGG ACTCCAGGAA CCCCCTGTGG
AAGGGCCCTG CCAAGCTGCT GTGGAAGGGG GAGGGGCTG TGGTGATCCA GGACAACTCT
GACATCAAGG TGGTGCCCAG GAGGAAGGCC AAGATCATCA GGGACTATGG CAAGCAGATG
GCTGGGGATG ACTGTGTGGC CTCCAGGCAG GATGAGGACT AAAGCCCGGG CAGATCT (SEQ ID
NO:3).

5

10

15

20

25

30

35

In order to produce the IA-pol-based adenoviral vaccines of the present invention, inactivation of the enzymatic functions was achieved by replacing a total of nine active site residues from the enzyme subunits with alanine side-chains. As shown in Table 1, all residues that comprise the catalytic triad of the polymerase, namely Asp112, Asp187, and Asp188, were substituted with alanine (Ala) residues (Larder, et al., Nature 1987, 327: 716-717; Larder, et al., 1989, Proc. Natl. Acad. Sci. 1989, 86: 4803-4807). Three additional mutations were introduced at Asp445, Glu480 and Asp500 to abolish RNase H activity (Asp551 was left unchanged in this IA Pol construct), with each residue being substituted for an Ala residue, respectively (Davies, et al., 1991, Science 252:, 88-95; Schatz, et al., 1989, FEBS Lett. 257: 311-314; Mizrahi, et al., 1990, Nucl. Acids. Res. 18: pp. 5359-5353). HIV pol integrase function was abolished through three mutations at Asp626, Asp678 and Glu714. Again, each of these residues has been substituted with an Ala residue (Wiskerchen, et al., 1995, J. Virol. 69: 376-386; Leavitt, et al., 1993, J. Biol. Chem. 268: 2113-2119). Amino acid residue Pro3 of SEQ ID NO:4 marks the start of the RT gene. The complete amino acid sequence of IA-Pol is disclosed herein as SEQ ID NO:4 and Figure 17A-C, as follows:

Met Ala Pro Ile Ser Pro Ile Glu Thr Val Pro Val Lys Leu Lys Pro Gly Met Asp Gly Pro Lys Val Lys Gln Trp Pro Leu Thr Glu Glu Lys Ile Lys Ala Leu Val Glu Ile Cys Thr Glu Met Glu Lys Glu Gly Lys Ile Ser Lys Ile Gly Pro Glu Asn Pro Tyr Asn Thr Pro Val Phe Ala Ile Lys Lys Lys Lys Asp Ser Thr Lys Trp Arg Lys Leu Val Asp Phe Arg

Glu Leu Asn Lys Arg Thr Gln Asp Phe Trp Glu Val Gln Leu Gly Ile Pro His Pro Ala Gly Leu Lys Lys Lys Ser Val Thr Val Leu Ala Val Gly Asp Ala Tyr Phe Ser Val Pro Leu Asp Glu Asp Phe Arg Lys Tyr Thr Ala Phe Thr Ile Pro Ser Ile Asn Asn Glu Thr Pro Gly Ile Arg Tyr Gln Tyr Asn Val Leu Pro Gln Gly Trp Lys Gly Ser Pro Ala Ile Phe Gln Ser Ser Met Thr Lys Ile Leu Glu Pro Phe Arg Lys Gln Asn Pro Asp Ile Val Ile Tyr Gln Tyr Met Ala Ala Leu Tyr Val Gly Ser Asp Leu Glu Ile Gly Gln His Arg Thr Lys Ile Glu Glu Leu Arg Gln His Leu Leu Arg Trp Gly Leu Thr Thr Pro Asp Lys Lys His Gln Lys Glu Pro Pro Phe Leu Trp Met Gly Tyr Glu Leu His Pro Asp Lys 10 Trp Thr Val Gln Pro Ile Val Leu Pro Glu Lys Asp Ser Trp Thr Val Asn Asp Ile Gln Lys Leu Val Gly Lys Leu Asn Trp Ala Ser Gln Ile Tyr Pro Gly Ile Lys Val Arg Gln Leu Cys Lys Leu Leu Arg Gly Thr Lys Ala Leu Thr Glu Val Ile Pro Leu Thr Glu Glu Ala Glu Leu Glu Leu Ala Glu Asn Arg Glu Ile Leu Lys Glu Pro Val His Gly Val Tyr 15 Tyr Asp Pro Ser Lys Asp Leu Ile Ala Glu Ile Gln Lys Gln Gly Gln Gly Gln Trp Thr Tyr Gln Ile Tyr Gln Glu Pro Phe Lys Asn Leu Lys Thr Gly Lys Tyr Ala Arg Met Arg Gly Ala His Thr Asn Asp Val Lys Gln Leu Thr Glu Ala Val Gln Lys Ile Thr Thr Glu Ser Ile Val Ile Trp Gly Lys Thr Pro Lys Phe Lys Leu Pro Ile Gln Lys Glu Thr Trp 20 Glu Thr Trp Trp Thr Glu Tyr Trp Gln Ala Thr Trp Ile Pro Glu Trp Glu Phe Val Asn Thr Pro Pro Leu Val Lys Leu Trp Tyr Gln Leu Glu Lys Glu Pro Ile Val Gly Ala Glu Thr Phe Tyr Val Ala Gly Ala Ala Asn Arg Glu Thr Lys Leu Gly Lys Ala Gly Tyr Val Thr Asn Arg Gly Arg Gln Lys Val Val Thr Leu Thr Asp Thr Thr Asn Gln Lys Thr Ala 25 Leu Gln Ala Ile Tyr Leu Ala Leu Gln Asp Ser Gly Leu Glu Val Asn Ile Val Thr Ala Ser Gln Tyr Ala Leu Gly Ile Ile Gln Ala Gln Pro Asp Gln Ser Glu Ser Glu Leu Val Asn Gln Ile Ile Glu Gln Leu Ile Lys Lys Glu Lys Val Tyr Leu Ala Trp Val Pro Ala His Lys Gly Ile Gly Gly Asn Glu Gln Val Asp Lys Leu Val Ser Ala Gly Ile Arg Lys . Val Leu Phe Leu Asp Gly Ile Asp Lys Ala Gln Asp Glu His Glu Lys Tyr His Ser Asn Trp Arg Ala Met Ala Ser Asp Phe Asn Leu Pro Pro Val Val Ala Lys Glu Ile Val Ala Ser Cys Asp Lys Cys Gln Leu Lys Gly Glu Ala Met His Gly Gln Val Asp Cys Ser Pro Gly Ile Trp Gln Leu Ala Cys Thr His Leu Glu Gly Lys Val Ile Leu Val Ala Val His 35 Val Ala Ser Gly Tyr Ile Glu Ala Glu Val Ile Pro Ala Glu Thr Gly

Gln Glu Thr Ala Tyr Phe Leu Leu Lys Leu Ala Gly Arg Trp Pro Val Lys Thr Ile His Thr Ala Asn Gly Ser Asn Phe Thr Gly Ala Thr Val Arg Ala Ala Cys Trp Trp Ala Gly Ile Lys Gln Glu Phe Gly Ile Pro Tyr Asn Pro Gln Ser Gln Gly Val Val Ala Ser Met Asn Lys Glu Leu Lys Lys Ile Ile Gly Gln Val Arg Asp Gln Ala Glu His Leu Lys Thr Ala Val Gln Met Ala Val Phe Ile His Asn Phe Lys Arg Lys Gly Gly Ile Gly Gly Tyr Ser Ala Gly Glu Arg Ile Val Asp Ile Ile Ala Thr Asp Ile Gln Thr Lys Glu Leu Gln Lys Gln Ile Thr Lys Ile Gln Asn Phe Arg Val Tyr Tyr Arg Asp Ser Arg Asn Pro Leu Trp Lys Gly Pro Ala Lys Leu Leu Trp Lys Gly Glu Gly Ala Val Val Ile Gln Asp Asn Ser Asp Ile Lys Val Val Pro Arg Arg Lys Ala Lys Ile Ile Arg Asp Tyr Gly Lys Gln Met Ala Gly Asp Asp Cys Val Ala Ser Arg Gln Asp Glu Asp (SEQ ID NO:4).

As noted above, it will be understood that any combination of the mutations disclosed above may be suitable and therefore be utilized as an IA-pol-based adenoviral HIV vaccine of the present invention, either when administered alone or in a combined modality regime and/or a prime-boost regimen. For example, it may be possible to mutate only 2 of the 3 residues within the respective reverse transcriptase, RNase-H, and integrase coding regions while still abolishing these enzymatic activities. However, the IA-pol construct described above and disclosed as SEQ ID NO:3, as well as the expressed protein (SEQ ID NO:4;) is preferred. It is also preferred that at least one mutation be present in each of the three catalytic domains.

Another aspect of this portion of the invention are codon optimized HIV-1 Pol-based vaccine constructions which comprise a eukaryotic trafficking signal peptide such as from tPA (tissue-type plasminogen activator) or by a leader peptide such as is found in highly expressed mammalian proteins such as immunoglobulin leader peptides. Any functional leader peptide may be tested for efficacy. However, a preferred embodiment of the present invention, as with HIV-1 Nef constructs shown herein, is to provide for a HIV-1 Pol mutant adenoviral vaccine construction wherein the pol coding region or a portion thereof is operatively linked to a leader peptide, preferably a leader peptide from human tPA. In other words, a codon optimized HIV-1 Pol mutant such as IA-Pol (SEQ ID NO:4) may also comprise a leader peptide at the amino terminal portion of the protein, which may effect cellular trafficking and hence, immunogenicity of the expressed protein within the host cell. As noted in Figure 16A-B, a DNA vector which may be utilized to practice the present invention may be modified by known recombinant DNA methodology to contain a leader signal

peptide of interest, such that downstream cloning of the modified HIV-1 protein of interest results in a nucleotide sequence which encodes a modified HIV-1 tPA/Pol protein. In the alternative, as noted above, insertion of a nucleotide sequence which encodes a leader peptide may be inserted into a DNA vector housing the open reading frame for the Pol protein of interest. Regardless of the cloning strategy, the end result is a polynucleotide vaccine which comprises vector components for effective gene expression in conjunction with nucleotide sequences which encode a modified HIV-1 Pol protein of interest, including but not limited to a HIV-1 Pol protein which contains a leader peptide. The amino acid sequence of the human tPA leader utilized herein is as follows: MDAMKRGLCCVLLLCGAVFVSPSEISS (SEQ ID NO:17). 10 Therefore, another aspect of the present invention is to generate HIV-1 Pol-based vaccine constructions which comprise a eukaryotic trafficking signal peptide such as from tPA. To this end, the present invention relates to a DNA molecule which encodes a codon optimized wt-pol DNA construct wherein the protease (PR) activity is deleted and a human tPA leader sequence is fused to the 5' end of the coding region. 15 A DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:5, the open reading frame disclosed herein as SEQ ID NO:6.

To this end, the present invention relates to a DNA molecule which encodes a codon optimized wt-pol DNA construct wherein the protease (PR) activity is deleted and a human tPA leader sequence is fused to the 5'end of the coding region (herein, "tPA-wt-pol"). A DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:5, the open reading frame being contained from an initiating Met residue at nucleotides 8-10 to a termination codon from nucleotides 2633-2635. SEQ ID NO:5 is as follows:

20

25

30

35

GATCACCATG GATGCAATGA AGAGAGGGCT CTGCTGTGTG CTGCTGCTGT GTGGAGCAGT
CTTCGTTTCG CCCAGCGAGA TCTCCGCCCC CATCTCCCCC ATTGAGACTG TGCCTGTGAA
GCTGAAGCCT GGCATGGATG GCCCCAAGGT GAAGCAGTGG CCCCTGACTG AGGAGAAGAT
CAAGGCCCTG GTGGAAATCT GCACTGAGAT GGAGAAGGAG GGCAAAATCT CCAAGATTGG
CCCCGAGAAC CCCTACAACA CCCCTGTGTT TGCCATCAAG AAGAAGGACT CCACCAAGTG
GAGGAAGCTG GTGGACTTCA GGGAGCTGAA CAAGAGGACC CAGGACTTCT GGGAGGTGCA
GCTGGGCATC CCCCACCCCG CTGGCCTGAA GAAGAAGAAG TCTGTGACTG TGCTGGATGT
GAGCGGATGCC TACTTCTCTG TGCCCCTGGA TGAGGACTTC AGGAAGTACA CTGCCTTCAC
CATCCCCTCC ATCAACAATG AGACCCCTGG CATCAGGTAC CAGTACAATG TGCTGCCCCA
GGGCTGGAAG GGCTCCCCTG CCATCTTCCA GTCCTCCATG ACCAAGATCC TGGAGCCCTT
CAGGAAGCAG AACCCTGACA TTGTGATCTA CCAGTACATG GATGACCTGT ATGTGGGCTC
TGACCTGGAG ATTGGGCAGC ACAGGACCAA GATTGAGGAG CTGAGGCAGC ACCTGCTGAG

GTGGGGCCTG ACCACCCCTG ACAAGAAGCA CCAGAAGGAG CCCCCCTTCC TGTGGATGGG CTATGAGCTG CACCCCGACA AGTGGACTGT GCAGCCCATT GTGCTGCCTG AGAAGGACTC CTGGACTGTG AATGACATCC AGAAGCTGGT GGGCAAGCTG AACTGGGCCT CCCAAATCTA CCCTGGCATC AAGGTGAGGC AGCTGTGCAA GCTGCTGAGG GGCACCAAGG CCCTGACTGA GGTGATCCCC CTGACTGAGG AGGCTGAGCT GGAGCTGGCT GAGAACAGGG AGATCCTGAA GGAGCCTGTG CATGGGGTGT ACTATGACCC CTCCAAGGAC CTGATTGCTG AGATCCAGAA GCAGGGCCAG GGCCAGTGGA CCTACCAAAT CTACCAGGAG CCCTTCAAGA ACCTGAAGAC TGGCAAGTAT GCCAGGATGA GGGGGGCCCA CACCAATGAT GTGAAGCAGC TGACTGAGGC TGTGCAGAAG ATCACCACTG AGTCCATTGT GATCTGGGGC AAGACCCCCA AGTTCAAGCT 10 GCCCATCCAG AAGGAGACCT GGGAGACCTG GTGGACTGAG TACTGGCAGG CCACCTGGAT CCCTGAGTGG GAGTTTGTGA ACACCCCCC CCTGGTGAAG CTGTGGTACC AGCTGGAGAA GGAGCCCATT GTGGGGGCTG AGACCTTCTA TGTGGATGGG GCTGCCAACA GGGAGACCAA GCTGGGCAAG GCTGGCTATG TGACCAACAG GGGCAGGCAG AAGGTGGTGA CCCTGACTGA CACCACCAAC CAGAAGACTG AGCTCCAGGC CATCTACCTG GCCCTCCAGG ACTCTGGCCT 15 GGAGGTGAAC ATTGTGACTG ACTCCCAGTA TGCCCTGGGC ATCATCCAGG CCCAGCCTGA TCAGTCTGAG TCTGAGCTGG TGAACCAGAT CATTGAGCAG CTGATCAAGA AGGAGAAGGT GTACCTGGCC TGGGTGCCTG CCCACAAGGG CATTGGGGGC AATGAGCAGG TGGACAAGCT GGTGTCTGCT GGCATCAGGA AGGTGCTGTT CCTGGATGGC ATTGACAAGG CCCAGGATGA GCATGAGAAG TACCACTCCA ACTGGAGGGC TATGGCCTCT GACTTCAACC TGCCCCCTGT 20 GGTGGCTAAG GAGATTGTGG CCTCCTGTGA CAAGTGCCAG CTGAAGGGGG AGGCCATGCA TGGGCAGGTG GACTGCTCCC CTGGCATCTG GCAGCTGGAC TGCACCCACC TGGAGGGCAA GGTGATCCTG GTGGCTGTGC ATGTGGCCTC CGGCTACATT GAGGCTGAGG TGATCCCTGC TGAGACAGGC CAGGAGACTG CCTACTTCCT GCTGAAGCTG GCTGGCAGGT GGCCTGTGAA GACCATCCAC ACTGACAATG GCTCCAACTT CACTGGGGCC ACAGTGAGGG CTGCCTGCTG 25 GTGGGCTGGC ATCAAGCAGG AGTTTGGCAT CCCCTACAAC CCCCAGTCCC AGGGGGTGGT GGAGTCCATG AACAAGGAGC TGAAGAAGAT CATTGGGCAG GTGAGGGACC AGGCTGAGCA CCTGAAGACA GCTGTGCAGA TGGCTGTTT CATCCACAAC TTCAAGAGGA AGGGGGGCAT CGGGGGCTAC TCCGCTGGGG AGAGGATTGT GGACATCATT GCCACAGACA TCCAGACCAA GGAGCTCCAG AAGCAGATCA CCAAGATCCA GAACTTCAGG GTGTACTACA GGGACTCCAG 30 GAACCCCTG TGGAAGGGCC CTGCCAAGCT GCTGTGGAAG GGGGAGGGGG CTGTGGTGAT CCAGGACAAC TCTGACATCA AGGTGGTGCC CAGGAGGAAG GCCAAGATCA TCAGGGACTA TGGCAAGCAG ATGGCTGGGG ATGACTGTGT GGCCTCCAGG CAGGATGAGG ACTAAAGCCC GGGCAGATCT (SEQ ID NO:5).

The open reading frame of the wild type tPA-pol construct disclosed as SEQ ID NO:5 contains 875 amino acids, disclosed herein as SEQ ID NO:6, as follows:

Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Cys Gly

35

Ala Val Phe Val Ser Pro Ser Glu Ile Ser Ala Pro Ile Ser Pro Ile Glu Thr Val Pro Val Lys Leu Lys Pro Gly Met Asp Gly Pro Lys Val Lys Gln Trp Pro Leu Thr Glu Glu Lys Ile Lys Ala Leu Val Glu Ile Cys Thr Glu Met Glu Lys Glu Gly Lys Ile Ser Lys Ile Gly Pro Glu Asn Pro Tyr Asn Thr Pro Val Phe Ala Ile Lys Lys Lys Asp Ser Thr Lys Trp Arg Lys Leu Val Asp Phe Arg Glu Leu Asn Lys Arg Thr Gln Asp Phe Trp Glu Val Gln Leu Gly Ile Pro His Pro Ala Gly Leu Lys Lys Lys Lys Ser Val Thr Val Leu Asp Val Gly Asp Ala Tyr Phe Ser Val Pro Leu Asp Glu Asp Phe Arg Lys Tyr Thr Ala Phe Thr Ile Pro Ser Ile Asn Asn Glu Thr Pro Gly Ile Arg Tyr Gln Tyr Asn Val Leu 10 Pro Gln Gly Trp Lys Gly Ser Pro Ala Ile Phe Gln Ser Ser Met Thr Lys Ile Leu Glu Pro Phe Arg Lys Gln Asn Pro Asp Ile Val Ile Tyr Gln Tyr Met Asp Asp Leu Tyr Val Gly Ser Asp Leu Glu Ile Gly Gln His Arg Thr Lys Ile Glu Glu Leu Arg Gln His Leu Leu Arg Trp Gly Leu Thr Thr Pro Asp Lys Lys His Gln Lys Glu Pro Pro Phe Leu Trp 15 Met Gly Tyr Glu Leu His Pro Asp Lys Trp Thr Val Gln Pro Ile Val Leu Pro Glu Lys Asp Ser Trp Thr Val Asn Asp Ile Gln Lys Leu Val Gly Lys Leu Asn Trp Ala Ser Gln Ile Tyr Pro Gly Ile Lys Val Arg Gln Leu Cys Lys Leu Leu Arg Gly Thr Lys Ala Leu Thr Glu Val Ile Pro Leu Thr Glu Glu Ala Glu Leu Glu Leu Ala Glu Asn Arg Glu Ile 20 Leu Lys Glu Pro Val His Gly Val Tyr Tyr Asp Pro Ser Lys Asp Leu Ile Ala Glu Ile Gln Lys Gln Gly Gln Gly Gln Trp Thr Tyr Gln Ile Tyr Gln Glu Pro Phe Lys Asn Leu Lys Thr Gly Lys Tyr Ala Arg Met Arg Gly Ala His Thr Asn Asp Val Lys Gln Leu Thr Glu Ala Val Gln Lys Ile Thr Thr Glu Ser Ile Val Ile Trp Gly Lys Thr Pro Lys Phe 25 Lys Leu Pro Ile Gln Lys Glu Thr Trp Glu Thr Trp Trp Thr Glu Tyr Trp Gln Ala Thr Trp Ile Pro Glu Trp Glu Phe Val Asn Thr Pro Pro Leu Val Lys Leu Trp Tyr Gln Leu Glu Lys Glu Pro Ile Val Gly Ala Glu Thr Phe Tyr Val Asp Gly Ala Ala Asn Arg Glu Thr Lys Leu Gly Lys Ala Gly Tyr Val Thr Asn Arg Gly Arg Gln Lys Val Val Thr Leu 30 Thr Asp Thr Thr Asn Gln Lys Thr.Glu Leu Gln Ala Ile Tyr Leu Ala Leu Gln Asp Ser Gly Leu Glu Val Asn Ile Val Thr Asp Ser Gln Tyr Ala Leu Gly Ile Ile Gln Ala Gln Pro Asp Gln Ser Glu Ser Glu Leu Val Asn Gln Ile Ile Glu Gln Leu Ile Lys Lys Glu Lys Val Tyr Leu Ala Trp Val Pro Ala His Lys Gly Ile Gly Gly Asn Glu Gln Val Asp 35 Lys Leu Val Ser Ala Gly Ile Arg Lys Val Leu Phe Leu Asp Gly Ile

Asp Lys Ala Gln Asp Glu His Glu Lys Tyr His Ser Asn Trp Arg Ala Met Ala Ser Asp Phe Asn Leu Pro Pro Val Val Ala Lys Glu Ile Val Ala Ser Cys Asp Lys Cys Gln Leu Lys Gly Glu Ala Met His Gly Gln Val Asp Cys Ser Pro Gly Ile Trp Gln Leu Asp Cys Thr His Leu Glu Gly Lys Val Ile Leu Val Ala Val His Val Ala Ser Gly Tyr Ile Glu Ala Glu Val Ile Pro Ala Glu Thr Gly Gln Glu Thr Ala Tyr Phe Leu Leu Lys Leu Ala Gly Arg Trp Pro Val Lys Thr Ile His Thr Asp Asn Gly Ser Asn Phe Thr Gly Ala Thr Val Arg Ala Ala Cys Trp Trp Ala Gly Ile Lys Gln Glu Phe Gly Ile Pro Tyr Asn Pro Gln Ser Gln Gly Val Val Glu Ser Met Asn Lys Glu Leu Lys Lys Ile Ile Gly Gln Val Arg Asp Gln Ala Glu His Leu Lys Thr Ala Val Gln Met Ala Val Phe Ile His Asn Phe Lys Arg Lys Gly Gly Ile Gly Gly Tyr Ser Ala Gly Glu Arg Ile Val Asp Ile Ile Ala Thr Asp Ile Gln Thr Lys Glu Leu Gln Lys Gln Ile Thr Lys Ile Gln Asn Phe Arg Val Tyr Tyr Arg Asp Ser Arg Asn Pro Leu Trp Lys Gly Pro Ala Lys Leu Leu Trp Lys Gly Glu Gly Ala Val Val Ile Gln Asp Asn Ser Asp Ile Lys Val Val Pro Arg Arg Lys Ala Lys Ile Ile Arg Asp Tyr Gly Lys Gln Met Ala Gly Asp Asp Cys Val Ala Ser Arg Gln Asp Glu Asp (SEQ ID NO:6).

15

20

25

30

35

The present invention also relates to a codon optimized HIV-1 Pol mutant contained within a recombinant adenoviral vector such as IA-Pol (SEQ ID NO:4) which comprises a leader peptide at the amino terminal portion of the protein, which may effect cellular trafficking and hence, immunogenicity of the expressed protein within the host cell. Any such adenoviral-based HIV-1 DNA pol mutant disclosed in the above paragraphs is suitable for fusion downstream of a leader peptide, such as a leader peptide including but not limited to the human tPA leader sequence. Therefore, any such leader peptide-based HIV-1 pol mutant construct may include but is not limited to a mutated DNA molecule which effectively alters the catalytic activity of the RT, RNase and/or IN region of the expressed protein, resulting in at least substantially decreased enzymatic activity one or more of the RT, RNase H and/or IN functions of HIV-1 Pol. In a preferred embodiment of this portion of the invention, a leader peptide/HIV-1 DNA pol construct contains a mutation or mutations within the Pol coding region which effectively abolishes RT, RNase H and IN activity. An especially preferable HIV-1 DNA pol construct is a DNA molecule which contains at least one point mutation which alters the active site and catalytic activity within the RT, RNase H and IN domains of Pol, such that each activity is at least substantially abolished, and preferably totally abolished. Such a HIV-1 Pol mutant will most likely

5

10

15

20

25

30

comprise at least one point mutation in or around each catalytic domain responsible for RT, RNase H and IN activity, respectfully. An especially preferred embodiment of this portion of the invention relates to a human tPA leader fused to the IA-Pol protein comprising the nine mutations shown in Table 1. The DNA molecule is disclosed herein as SEQ ID NO:7 and the expressed tPA-IA Pol protein comprises a fusion junction as shown in Figure 18. The complete amino acid sequence of the expressed protein is set forth in SEQ ID NO:8. To this end, SEQ ID NO:7 discloses the nucleotide sequence which codes for a human tPA leader fused to the IA Pol protein comprising the nine mutations shown in Table 1 (herein, "tPA-opt-IApol"). The open reading frame begins with the initiating Met (nucleotides 8-10) and terminates with a "TAA" codon at nucleotides 2633-2635. The nucleotide sequence encoding tPA-IAPol is also disclosed as follows: GATCACCATG GATGCAATGA AGAGAGGGCT CTGCTGTGTG CTGCTGCTGT GTGGAGCAGT CTTCGTTTCG CCCAGCGAGA TCTCCGCCCC CATCTCCCCC ATTGAGACTG TGCCTGTGAA GCTGAAGCCT GGCATGGATG GCCCCAAGGT GAAGCAGTGG CCCCTGACTG AGGAGAAGAT CAAGGCCCTG GTGGAAATCT GCACTGAGAT GGAGAAGGAG GGCAAAATCT CCAAGATTGG CCCCGAGAAC CCCTACAACA CCCCTGTGTT TGCCATCAAG AAGAAGGACT CCACCAAGTG GAGGAAGCTG GTGGACTTCA GGGAGCTGAA CAAGAGGACC CAGGACTTCT GGGAGGTGCA GCTGGGCATC CCCCACCCG CTGGCCTGAA GAAGAAGAAG TCTGTGACTG TGCTGGCTGT GGGGGATGCC TACTTCTCTG TGCCCCTGGA TGAGGACTTC AGGAAGTACA CTGCCTTCAC CATCCCCTCC ATCAACAATG AGACCCCTGG CATCAGGTAC CAGTACAATG TGCTGCCCCA GGGCTGGAAG GGCTCCCCTG CCATCTTCCA GTCCTCCATG ACCAAGATCC TGGAGCCCTT CAGGAAGCAG AACCCTGACA TTGTGATCTA CCAGTACATG GCTGCCCTGT ATGTGGGCTC TGACCTGGAG ATTGGGCAGC ACAGGACCAA GATTGAGGAG CTGAGGCAGC ACCTGCTGAG GTGGGGCCTG ACCACCCCTG ACAAGAAGCA CCAGAAGGAG CCCCCCTTCC TGTGGATGGG CTATGAGCTG CACCCCGACA AGTGGACTGT GCAGCCCATT GTGCTGCCTG AGAAGGACTC CTGGACTGTG AATGACATCC AGAAGCTGGT GGGCAAGCTG AACTGGGCCT CCCAAATCTA CCCTGGCATC AAGGTGAGGC AGCTGTGCAA GCTGCTGAGG GGCACCAAGG CCCTGACTGA GGTGATCCCC CTGACTGAGG AGGCTGAGCT GGAGCTGGCT GAGAACAGGG AGATCCTGAA GGAGCCTGTG CATGGGGTGT ACTATGACCC CTCCAAGGAC CTGATTGCTG AGATCCAGAA -GCAGGGCCAG GGCCAGTGGA CCTACCAAAT CTACCAGGAG CCCTTCAAGA ACCTGAAGAC TGGCAAGTAT GCCAGGATGA GGGGGGCCCA CACCAATGAT GTGAAGCAGC TGACTGAGGC TGTGCAGAAG ATCACCACTG AGTCCATTGT GATCTGGGGC AAGACCCCCA AGTTCAAGCT GCCCATCCAG AAGGAGACCT GGGAGACCTG GTGGACTGAG TACTGGCAGG CCACCTGGAT CCCTGAGTGG GAGTTTGTGA ACACCCCCC CCTGGTGAAG CTGTGGTACC AGCTGGAGAA GGAGCCCATT GTGGGGGCTG AGACCTTCTA TGTGGCTGGG GCTGCCAACA GGGAGACCAA

GCTGGGCAAG GCTGGCTATG TGACCAACAG GGGCAGGCAG AAGGTGGTGA CCCTGACTGA CACCACCAAC CAGAAGACTG CCCTCCAGGC CATCTACCTG GCCCTCCAGG ACTCTGGCCT GGAGGTGAAC ATTGTGACTG CCTCCCAGTA TGCCCTGGGC ATCATCCAGG CCCAGCCTGA TCAGTCTGAG TCTGAGCTGG TGAACCAGAT CATTGAGCAG CTGATCAAGA AGGAGAAGGT GTACCTGGCC TGGGTGCCTG CCCACAAGGG CATTGGGGGC AATGAGCAGG TGGACAAGCT GGTGTCTGCT GGCATCAGGA AGGTGCTGTT CCTGGATGGC ATTGACAAGG CCCAGGATGA GCATGAGAAG TACCACTCCA ACTGGAGGGC TATGGCCTCT GACTTCAACC TGCCCCCTGT GGTGGCTAAG GAGATTGTGG CCTCCTGTGA CAAGTGCCAG CTGAAGGGGG AGGCCATGCA TGGGCAGGTG GACTGCTCCC CTGGCATCTG GCAGCTGGCC TGCACCCACC TGGAGGGCAA GGTGATCCTG GTGGCTGTGC ATGTGGCCTC CGGCTACATT GAGGCTGAGG TGATCCCTGC TGAGACAGGC CAGGAGACTG CCTACTTCCT GCTGAAGCTG GCTGGCAGGT GGCCTGTGAA GACCATCCAC ACTGCCAATG GCTCCAACTT CACTGGGGCC ACAGTGAGGG CTGCCTGCTG GTGGGCTGGC ATCAAGCAGG AGTTTGGCAT CCCCTACAAC CCCCAGTCCC AGGGGGTGGT GGCCTCCATG AACAAGGAGC TGAAGAAGAT CATTGGGCAG GTGAGGGACC AGGCTGAGCA CCTGAAGACA GCTGTGCAGA TGGCTGTGTT CATCCACAAC TTCAAGAGGA AGGGGGGCAT CGGGGGCTAC TCCGCTGGGG AGAGGATTGT GGACATCATT GCCACAGACA TCCAGACCAA GGAGCTCCAG AAGCAGATCA CCAAGATCCA GAACTTCAGG GTGTACTACA GGGACTCCAG GAACCCCCTG TGGAAGGGCC CTGCCAAGCT GCTGTGGAAG GGGGAGGGGG CTGTGGTGAT CCAGGACAAC TCTGACATCA AGGTGGTGCC CAGGAGGAAG GCCAAGATCA TCAGGGACTA TGGCAAGCAG ATGGCTGGGG ATGACTGTGT GGCCTCCAGG CAGGATGAGG ACTAAAGCCC GGGCAGATCT (SEQ ID NO:7).

10

15

20

The open reading frame of the tPA-IA-pol construct disclosed as SEQ ID NO:7 contains 875 amino acids, disclosed herein as tPA-IA-Pol and SEQ ID NO:8, as follows:

25 Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly Ala Val Phe Val Ser Pro Ser Glu Ile Ser Ala Pro Ile Ser Pro Ile Glu Thr Val Pro Val Lys Leu Lys Pro Gly Met Asp Gly Pro Lys Val Lys Gln Trp Pro Leu Thr Glu Glu Lys Ile Lys Ala Leu Val Glu Ile Cys Thr Glu Met Glu Lys Glu Gly Lys Ile Ser Lys Ile Gly Pro Glu 30 Asn Pro Tyr Asn Thr Pro Val Phe Ala Ile Lys Lys Lys Asp Ser Thr Lys Trp Arg Lys Leu Val Asp Phe Arg Glu Leu Asn Lys Arg Thr Gln Asp Phe Trp Glu Val Gln Leu Gly Ile Pro His Pro Ala Gly Leu Lys Lys Lys Lys Ser Val Thr Val Leu Ala Val Gly Asp Ala Tyr Phe Ser Val Pro Leu Asp Glu Asp Phe Arg Lys Tyr Thr Ala Phe Thr Ile Pro Ser Ile Asn Asn Glu Thr Pro Gly Ile Arg Tyr Gln Tyr Asn Val Leu Pro Gln Gly Trp Lys Gly Ser Pro Ala Ile Phe Gln Ser Ser Met Thr

Lys Ile Leu Glu Pro Phe Arg Lys Gln Asn Pro Asp Ile Val Ile Tyr Gln Tyr Met Ala Ala Leu Tyr Val Gly Ser Asp Leu Glu Ile Gly Gln His Arg Thr Lys Ile Glu Glu Leu Arg Gln His Leu Leu Arg Trp Gly Leu Thr Thr Pro Asp Lys Lys His Gln Lys Glu Pro Pro Phe Leu Trp Met Gly Tyr Glu Leu His Pro Asp Lys Trp Thr Val Gln Pro Ile Val Leu Pro Glu Lys Asp Ser Trp Thr Val Asn Asp Ile Gln Lys Leu Val Gly Lys Leu Asn Trp Ala Ser Gln Ile Tyr Pro Gly Ile Lys Val Arg Gln Leu Cys Lys Leu Leu Arg Gly Thr Lys Ala Leu Thr Glu Val Ile Pro Leu Thr Glu Glu Ala Glu Leu Glu Leu Ala Glu Asn Arg Glu Ile Leu Lys Glu Pro Val His Gly Val Tyr Tyr Asp Pro Ser Lys Asp Leu 10 Ile Ala Glu Ile Gln Lys Gln Gly Gln Gly Gln Trp Thr Tyr Gln Ile Tyr Gln Glu Pro Phe Lys Asn Leu Lys Thr Gly Lys Tyr Ala Arg Met Arg Gly Ala His Thr Asn Asp Val Lys Gln Leu Thr Glu Ala Val Gln Lys Ile Thr Thr Glu Ser Ile Val Ile Trp Gly Lys Thr Pro Lys Phe Lys Leu Pro Ile Gln Lys Glu Thr Trp Glu Thr Trp Trp Thr Glu Tyr 15 Trp Gln Ala Thr Trp Ile Pro Glu Trp Glu Phe Val Asn Thr Pro Pro Leu Val Lys Leu Trp Tyr Gln Leu Glu Lys Glu Pro Ile Val Gly Ala Glu Thr Phe Tyr Val Ala Gly Ala Ala Asn Arg Glu Thr Lys Leu Gly Lys Ala Gly Tyr Val Thr Asn Arg Gly Arg Gln Lys Val Val Thr Leu Thr Asp Thr Thr Asn Gln Lys Thr Ala Leu Gln Ala Ile Tyr Leu Ala 20 Leu Gln Asp Ser Gly Leu Glu Val Asn Ile Val Thr Ala Ser Gln Tyr Ala Leu Gly Ile Ile Gln Ala Gln Pro Asp Gln Ser Glu Ser Glu Leu Val Asn Gln Ile Ile Glu Gln Leu Ile Lys Lys Glu Lys Val Tyr Leu Ala Trp Val Pro Ala His Lys Gly Ile Gly Gly Asn Glu Gln Val Asp Lys Leu Val Ser Ala Gly Ile Arg Lys Val Leu Phe Leu Asp Gly Ile 25 Asp Lys Ala Gln Asp Glu His Glu Lys Tyr His Ser Asn Trp Arg Ala Met Ala Ser Asp Phe Asn Leu Pro Pro Val Val Ala Lys Glu Ile Val Ala Ser Cys Asp Lys Cys Gln Leu Lys Gly Glu Ala Met His Gly Gln Val Asp Cys Ser Pro Gly Ile Trp Gln Leu Ala Cys Thr His Leu Glu Gly Lys Val Ile Leu Val Ala Val His Val Ala Ser Gly Tyr Ile Glu 30 Ala Glu Val Ile Pro Ala Glu Thr Gly Gln Glu Thr Ala Tyr Phe. Leu Leu Lys Leu Ala Gly Arg Trp Pro Val Lys Thr Ile His Thr Ala Asn Gly Ser Asn Phe Thr Gly Ala Thr Val Arg Ala Ala Cys Trp Trp Ala Gly Ile Lys Gln Glu Phe Gly Ile Pro Tyr Asn Pro Gln Ser Gln Gly Val Val Ala Ser Met Asn Lys Glu Leu Lys Lys Ile Ile Gly Gln Val 35 Arg Asp Gln Ala Glu His Leu Lys Thr Ala Val Gln Met Ala Val Phe

Ile His Asn Phe Lys Arg Lys Gly Gly Ile Gly Gly Tyr Ser Ala Gly Glu Arg Ile Val Asp Ile Ile Ala Thr Asp Ile Gln Thr Lys Glu Leu Gln Lys Gln Ile Thr Lys Ile Gln Asn Phe Arg Val Tyr Tyr Arg Asp Ser Arg Asn Pro Leu Trp Lys Gly Pro Ala Lys Leu Leu Trp Lys Gly Glu Gly Ala Val Val Ile Gln Asp Asn Ser Asp Ile Lys Val Val Pro Arg Arg Lys Ala Lys Ile Ile Arg Asp Tyr Gly Lys Gln Met Ala Gly Asp Asp Cys Val Ala Ser Arg Gln Asp Glu Asp (SEQ ID NO:8).

EXAMPLE 18

CODON OPTIMIZED HIV-1 NEF AND CODON OPTIMIZED HIV-1 NEF MODIFICATIONS

10

15

20

25

30

35

Codon optimized version of HIV-1 Nef and HIV-1 Nef modifications are essentially as described in U.S. Application Serial No. 09/738,782, filed December 15, 2000 and PCT International Application PCT/US00/34162, also filed December 15, 2000, both documents which are hereby incorporated by reference. As disclosed within the above-mentioned documents, particular embodiments of codon optimized Nef and Nef modifications relate to a DNA molecule encoding HIV-1 Nef from the HIV-1 ifrl isolate wherein the codons are optimized for expression in a mammalian system such as a human. The DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:9, while the expressed open reading frame is disclosed herein as SEQ ID NO:10. Another embodiment of Nef-based coding regions for use in the adenoviral vectors of the present invention comprise a codon optimized DNA molecule encoding a protein containing the human plasminogen activator (tpa) leader peptide fused with the NH2-terminus of the HIV-1 Nef polypeptide. The DNA molecule which encodes this protein is disclosed herein as SEO ID NO:11, while the expressed open reading frame is disclosed herein as SEQ ID NO:12. Another modified Nef optimized coding region relates to a DNA molecule encoding optimized HIV-1 Nef wherein the open reading frame codes for modifications at the amino terminal myristylation site (Gly-2 to Ala-2) and substitution of the Leu-174-Leu-175 dileucine motif to Ala-174-Ala-175, herein described as opt nef (G2A, LLAA). The DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:13, while the expressed open reading frame is disclosed herein as SEQ ID NO:14. An additional embodiment relates to a DNA molecule encoding optimized HIV-1 Nef wherein the amino terminal myristylation site and dileucine motif have been deleted, as well as comprising a tPA leader peptide. This DNA molecule, opt tpanef (LLAA), comprises an open reading frame which

encodes a Nef protein containing a tPA leader sequence fused to amino acid residue 6-216 of HTV-1 Nef (ifrl), wherein Leu-174 and Leu-175 are substituted with Ala-174 and Ala-175, herein referred to as opt tpanef (LLAA) is disclosed herein as SEQ ID NO:15, while the expressed open reading frame is disclosed herein as SEQ ID NO:16.

As disclosed in the above-identified documents (U.S. Application Serial No. 09/738,782 and PCT International Application PCT/US00/34162) and reiterated herein, the following nef-based nucleotide and amino acid sequences which comprise the respective open reading frame are as follows:

5

10

15

20

25

30

The nucleotide sequence of the codon optimized version of HIV-1 jrfl nef gene is disclosed herein as SEQ ID NO:9, as shown herein: GATCTGCCAC CATGGGCGGC AAGTGGTCCA AGAGGTCCGT GCCCGGCTGG TCCACCGTGA GGGAGAGGAT GAGGAGGGCC GAGCCCGCCG CCGACAGGGT GAGGAGGACC GAGCCCGCCG CCGTGGCCGT GGGCGCCGTG TCCAGGGACC TGGAGAAGCA CGGCGCCATC ACCTCCTCCA ACACCGCCGC CACCAACGCC GACTGCGCCT GGCTGGAGGC CCAGGAGGAC GAGGAGGTGG GCTTCCCCGT GAGGCCCCAG GTGCCCCTGA GGCCCATGAC CTACAAGGGC GCCGTGGACC TGTCCCACTT CCTGAAGGAG AAGGGCGGCC TGGAGGGCCT GATCCACTCC CAGAAGAGGC AGGACATCCT GGACCTGTGG GTGTACCACA CCCAGGGCTA CTTCCCCGAC TGGCAGAACT ACACCCCGG CCCCGGCATC AGGTTCCCCC TGACCTTCGG CTGGTGCTTC AAGCTGGTGC CCGTGGAGCC CGAGAAGGTG GAGGAGGCCA ACGAGGGCGA GAACAACTGC CTGCTGCACC CCATGTCCCA GCACGGCATC GAGGACCCCG AGAAGGAGGT GCTGGAGTGG AGGTTCGACT CCAAGCTGGC CTTCCACCAC GTGGCCAGGG AGCTGCACCC CGAGTACTAC AAGGACTGCT AAAGCCCGGG C (SEQ ID NO:9).

Preferred codon usage is as follows: Met (ATG), Gly (GGC), Lys (AAG), Trp (TGG), Ser (TCC), Arg (AGG), Val (GTG), Pro (CCC), Thr (ACC), Glu (GAG); Leu (CTG), His (CAC), Ile (ATC), Asn (AAC), Cys (TGC), Ala (GCC), Gln (CAG), Phe (TTC) and Tyr (TAC). For an additional discussion relating to mammalian (human) codon optimization, see WO 97/31115 (PCT/US97/02294), which is hereby incorporated by reference. See also Figure 19A-B for a comparion of wild type vs. codon optimized nucleotides comprising the open reading frame of HIV-Nef.

The open reading frame for SEQ ID NO:9 above comprises an initiating methionine residue at nucleotides 12-14 and a "TAA" stop codon from nucleotides 660-662. The open reading frame of SEQ ID NO:9 provides for a 216 amino acid HIV-1 Nef protein expressed through utilization of a codon optimized DNA vaccine vector. The 216 amino acid HIV-1 Nef (ifrl) protein is disclosed herein as SEQ ID 35 NO:10, and as follows:

Met Gly Gly Lys Trp Ser Lys Arg Ser Val Pro Gly Trp Ser Thr Val

Arg Glu Arg Met Arg Arg Ala Glu Pro Ala Ala Asp Arg Val Arg Arg
Thr Glu Pro Ala Ala Val Gly Val Gly Ala Val Ser Arg Asp Leu Glu
Lys His Gly Ala Ile Thr Ser Ser Asn Thr Ala Ala Thr Asn Ala Asp
Cys Ala Trp Leu Glu Ala Gln Glu Asp Glu Glu Val Gly Phe Pro Val

5 Arg Pro Gln Val Pro Leu Arg Pro Met Thr Tyr Lys Gly Ala Val Asp
Leu Ser His Phe Leu Lys Glu Lys Gly Gly Leu Glu Gly Leu Ile His
Ser Gln Lys Arg Gln Asp Ile Leu Asp Leu Trp Val Tyr His Thr Gln
Gly Tyr Phe Pro Asp Trp Gln Asn Tyr Thr Pro Gly Pro Gly Ile Arg
Phe Pro Leu Thr Phe Gly Trp Cys Phe Lys Leu Val Pro Val Glu Pro
Glu Lys Val Glu Glu Ala Asn Glu Gly Glu Asn Asn Cys Leu His
Pro Met Ser Gln His Gly Ile Glu Asp Pro Glu Lys Glu Val Leu Glu
Trp Arg Phe Asp Ser Lys Leu Ala Phe His His Val Ala Arg Glu Leu
His Pro Glu Tyr Tyr Lys Asp Cys (SEQ ID NO:10).

15

20

25

30

35

HIV-1 Nef is a 216 amino acid cytosolic protein which associates with the inner surface of the host cell plasma membrane through myristylation of Gly-2 (Franchini et al., 1986, Virology 155: 593-599). While not all possible Nef functions have been elucidated, it has become clear that correct trafficking of Nef to the inner plasma membrane promotes viral replication by altering the host intracellular environment to facilitate the early phase of the HIV-1 life cycle and by increasing the infectivity of progeny viral particles. In one aspect of the invention regarding codon-optimized, protein-modified polypeptides, the nef-encoding region of the adenovirus vector of the present invention is modified to contain a nucleotide sequence which encodes a heterologous leader peptide such that the amino terminal region of the expressed protein will contain the leader peptide. The diversity of function that typifies eukaryotic cells depends upon the structural differentiation of their membrane boundaries. To generate and maintain these structures, proteins must be transported from their site of synthesis in the endoplasmic reticulum to predetermined destinations throughout the cell. This requires that the trafficking proteins display sorting signals that are recognized by the molecular machinery responsible for route selection located at the access points to the main trafficking pathways. Sorting decisions for most proteins need to be made only once as they traverse their biosynthetic pathways since their final destination, the cellular location at which they perform their function, becomes their permanent residence. Maintenance of intracellular integrity depends in part on the selective sorting and accurate transport of proteins to their correct destinations. Defined sequence motifs exist in proteins which can act as 'address labels'. A number of sorting signals have

been found associated with the cytoplasmic domains of membrane proteins. An effective induction of CTL responses often required sustained, high level endogenous expression of an antigen. As membrane-association via myristylation is an essential requirement for most of Nef's function, mutants lacking myristylation, by glycine-to-alanine change, change of the dileucine motif and/or by substitution with a tpa leader sequence as described herein, will be functionally defective, and therefore will have improved safety profile compared to wild-type Nef for use as an HIV-1 vaccine component.

In another embodiment of this portion of the invention, either the DNA vector or the HIV-1 nef nucleotide sequence is modified to include the human tissue-specific plasminogen activator (tPA) leader. As shown in Figure 16A-B, a DNA vector may be modified by known recombinant DNA methodology to contain a leader signal peptide of interest, such that downstream cloning of the modified HIV-1 protein of interest results in a nucleotide sequence which encodes a modified HIV-1 tPA/Nef protein. In the alternative, as noted above, insertion of a nucleotide sequence which encodes a leader peptide may be inserted into a DNA vector housing the open reading frame for the Nef protein of interest. Regardless of the cloning strategy, the end result is a polynucleotide vaccine which comprises vector components for effective gene expression in conjunction with nucleotide sequences which encode a modified HIV-1 Nef protein of interest, including but not limited to a HIV-1 Nef protein which contains a leader peptide. The amino acid sequence of the human tPA leader utilized herein is as follows: MDAMKRGLCCVLLLCGAVFVSPSEISS (SEQ ID NO:17).

It has been shown that myristylation of Gly-2 in conjunction with a dileucine motif in the carboxy region of the protein is essential for Nef-induced down regulation of CD4 (Aiken et al., 1994, Cell 76: 853-864) via endocytosis. It has also been shown that Nef expression promotes down regulation of MHCI (Schwartz et al., 1996, Nature Medicine 2(3): 338-342) via endocytosis. The present invention relates in part to DNA vaccines which encode modified Nef proteins altered in trafficking and/or functional properties. The modifications introduced into the adenoviral vector HIV vaccines of the present invention include but are not limited to additions, deletions or substitutions to the nef open reading frame which results in the expression of a modified Nef protein which includes an amino terminal leader peptide, modification or deletion of the amino terminal myristylation site, and modification or deletion of the dileucine motif within the Nef protein and which alter function within the infected host cell. Therefore, a central theme of the DNA molecules and recombinant adenoviral HIV vaccines of the present invention is (1)

host administration and intracellular delivery of a codon optimized nef-based adenoviral HIV vaccine; (2) expression of a modified Nef protein which is immunogenic in terms of eliciting both CTL and Th responses; and, (3) inhibiting or at least altering known early viral functions of Nef which have been shown to promote HIV-1 replication and load within an infected host. Therefore, the nef coding region may be altered, resulting in a DNA vaccine which expresses a modified Nef protein wherein the amino terminal Gly-2 myristylation residue is either deleted or modified to express alternate amino acid residues. Also, the nef coding region may be altered so as to result in a DNA vaccine which expresses a modified Nef protein wherein the dileucine motif is either deleted or modified to express alternate amino acid residues. In addition, the adenoviral vector HIV vaccines of the present invention also relate to an isolated DNA molecule, regardless of codon usage, which expresses a wild type or modified Nef protein as described herein, including but not limited to modified Nef proteins which comprise a deletion or substitution of Gly 2, a deletion or substitution of Leu 174 and Leu 175 and/or inclusion of a leader sequence.

10

15

20

25

30

35

Therefore, specific Nef-based constructs further include the following, as exemplification's and not limitations. For example, the present invention relates to an adenoviral vector vaccine which encodes modified forms of HIV-1, an open reading frame which encodes a Nef protein which comprises a tPA leader sequence fused to amino acid residue 6-216 of HIV-1 Nef (jfrl) is referred to herein as opt tpanef. The nucleotide sequence comprising the open reading frame of opt tpanef is disclosed herein as SEQ ID NO:11, as shown below:

CATGGATGCA ATGAAGAGA GGCTCTGCTG TGTGCTGCTG CTGTGGAG CAGTCTTCGT
TTCGCCCAGC GAGATCTCCT CCAAGAGGTC CGTGCCCGGC TGGTCCACCG TGAGGGAGAG
GATGAGGAGG GCCGAGCCCG CCGCCGACAG GGTGAGGAGG ACCGAGCCCG CCGCCGTGGG
CGTGGGCGCC GTGTCCAGGG ACCTGGAGAA GCACGGCGCC ATCACCTCCT CCAACACCGC
CGCCACCAAC GCCGACTGCG CCTGGCTGGA GGCCCAGGAG GACGAGGAGG TGGGCTTCCC
CGTGAGGCCC CAGGTGCCCC TGAGGCCCAT GACCTACAAG GGCGCCGTGG ACCTGTCCCA
CTTCCTGAAG GAGAAGGGCG GCCTGGAGGG CCTGATCCAC TCCCAGAAGA GGCAGGACAT
CCTGGACCTG TGGGTGTACC ACACCCAGGG CTACTTCCCC GACTGGCAGA ACTACACCCC
CGGCCCCGGC ATCAGGTTCC CCCTGACCTT CGGCTGGTGC TTCAAGCTGG TGCCCGTGGA
GCCCGAGAAG GTGGAGGAGG CCAACGAGGG CGAGAACAAC TGCCTGCTGC ACCCCATGTC
CCAGCACGGC ATCGAGGACC CCGAGAAGGA GGTGCTGGAG TGGAGGTTCG ACCCCATGTC
GGCCTTCCAC CACGTGGCCA GGGAGCTGCA CCCCGAGTAC TACAAGGACT GCTAAAGCC
(SEQ ID NO:11).

The open reading frame for SEQ ID NO:11 comprises an initiating methionine

residue at nucleotides 2-4 and a "TAA" stop codon from nucleotides 713-715. The open reading frame of SEQ ID NO:3 provides for a 237 amino acid HIV-1 Nef protein which comprises a tPA leader sequence fused to amino acids 6-216 of HIV-1 Nef, including the dileucine motif at amino acid residues 174 and 175. This 237 amino acid tPA/Nef (jfrl) fusion protein is disclosed herein as SEQ ID NO:12, and is shown as follows:

Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Cys Gly Ala Val Phe Val Ser Pro Ser Glu Ile Ser Ser Lys Arg Ser Val Pro Gly Trp Ser Thr Val Arg Glu Arg Met Arg Arg Ala Glu Pro Ala Ala Asp Arg Val Arg Arg Thr Glu Pro Ala Ala Val Gly Val Gly Ala Val Ser Arg Asp Leu Glu Lys His Gly Ala Ile Thr Ser Ser Asn Thr Ala Ala Thr Asn Ala Asp Cys Ala Trp Leu Glu Ala Gln Glu Asp Glu Glu Val Gly Phe Pro Val Arg Pro Gln Val Pro Leu Arg Pro Met Thr Tyr Lys Gly Ala Val Asp Leu Ser His Phe Leu Lys Glu Lys Gly Gly Leu Glu Gly Leu Ile His Ser Gln Lys Arg Gln Asp Ile Leu Asp Leu Trp Val Tyr His Thr Gln Gly Tyr Phe Pro Asp Trp Gln Asn Tyr Thr Pro Gly Pro Gly Ile Arg Phe Pro Leu Thr Phe Gly Trp Cys Phe Lys Leu Val Pro Val Glu Pro Glu Lys Val Glu Glu Ala Asn Glu Gly Glu Asn Asn Cys Leu Leu His Pro Met Ser Gln His Gly Ile Glu Asp Pro Glu Lys Glu Val Leu Glu Trp Arg Phe Asp Ser Lys Leu Ala Phe His His Val Ala Arg Glu Leu His Pro Glu Tyr Tyr Lys Asp Cys (SEQ ID NO:12). Therefore, this exemplified Nef protein, Opt tPA-Nef, contains both a tPA leader sequence as well as deleting the myristylation site of Gly-2A DNA molecule encoding HIV-1 Nef from the HIV-1 jfrl isolate wherein the codons are optimized for expression in a mammalian system such as a human.

In another specific embodiment of the present invention, a DNA molecule is disclosed which encodes optimized HIV-1 Nef wherein the open reading frame of a recombinant adenoviral HIV vaccine encodes for modifications at the amino terminal myristylation site (Gly-2 to Ala-2) and substitution of the Leu-174-Leu-175 dileucine motif to Ala-174-Ala-175. This open reading frame is herein described as opt nef (G2A,LLAA) and is disclosed as SEQ ID NO:13, which comprises an initiating methionine residue at nucleotides 12-14 and a "TAA" stop codon from nucleotides 660-662. The nucleotide sequence of this codon optimized version of HIV-1 jrfl nef gene with the above mentioned modifications is disclosed herein as SEQ ID NO:13,

35 as follows:

10

15

20

25

30

GATCTGCCAC CATGGCCGGC AAGTGGTCCA AGAGGTCCGT GCCCGGCTGG TCCACCGTGA
GGGAGAGGAT GAGGAGGGCC GAGCCCGCCG CCGACAGGGT GAGGAGGACC GAGCCCGCCG
CCGTGGGCGT GGGCGCCGTG TCCAGGGACC TGGAGAAGCA CGGCGCCATC ACCTCCTCA
ACACCGCCGC CACCAACGCC GACTGCGCCT GGCTGGAGGC CCAGGAGGAC GAGGAGGTGG
GCTTCCCCGT GAGGCCCCAG GTGCCCCTGA GGCCCATGAC CTACAAGGGC GCCGTGGACC
TGTCCCACTT CCTGAAGGAG AAGGGCGGCC TGGAGGGCCT GATCCACTCC CAGAAGAGGC
AGGACATCCT GGACCTGTGG GTGTACCACA CCCAGGGCTA CTTCCCCGAC TGGCAGAACT
ACACCCCCGG CCCCGGCATC AGGTTCCCCC TGACCTTCGG CTGGTGCTTC AAGCTGGTGC
CCGTGGAGCC CGAGAAGGTG GAGGAGCCCA ACGAGGGCGA GAACAACTGC GCCGCCCACC
CCATGTCCCA GCACGGCATC GAGGACCCCG AGAAGGAGGT GCTGGAGTGG AGGTTCGACT
CCAAGCTGGC CTTCCACCAC GTGGCCAGGG AGCTGCACCC CGAGTACTAC AAGGACTGCT
AAAGCCCGGG C (SEQ ID NO:13).

The open reading frame of SEQ ID NO:13 encodes Nef (G2A,LLAA), disclosed herein as SEQ ID NO:14, as follows:

10

30

35

Met Ala Gly Lys Trp Ser Lys Arg Ser Val Pro Gly Trp Ser Thr Val 15 Arg Glu Arg Met Arg Arg Ala Glu Pro Ala Ala Asp Arg Val Arg Arg Thr Glu Pro Ala Ala Val Gly Val Gly Ala Val Ser Arg Asp Leu Glu Lys His Gly Ala Ile Thr Ser Ser Asn Thr Ala Ala Thr Asn Ala Asp Cys Ala Trp Leu Glu Ala Gln Glu Asp Glu Glu Val Gly Phe Pro Val Arg Pro Gln Val Pro Leu Arg Pro Met Thr Tyr Lys Gly Ala Val Asp 20 Leu Ser His Phe Leu Lys Glu Lys Gly Gly Leu Glu Gly Leu Ile His Ser Gln Lys Arg Gln Asp Ile Leu Asp Leu Trp Val Tyr His Thr Gln Gly Tyr Phe Pro Asp Trp Gln Asn Tyr Thr Pro Gly Pro Gly Ile Arg Phe Pro Leu Thr Phe Gly Trp Cys Phe Lys Leu Val Pro Val Glu Pro Glu Lys Val Glu Glu Ala Asn Glu Gly Glu Asn Asn Cys Ala Ala His 25 Pro Met Ser Gln His Gly Ile Glu Asp Pro Glu Lys Glu Val Leu Glu Trp Arg Phe Asp Ser Lys Leu Ala Phe His His Val Ala Arg Glu Leu His Pro Glu Tyr Tyr Lys Asp Cys Ser (SEQ ID NO:14).

An additional embodiment of the present invention relates to another DNA molecule encoding optimized HIV-1 Nef wherein the amino terminal myristylation site and dileucine motif have been deleted, as well as comprising a tPA leader peptide. This DNA molecule, opt tpanef (LLAA) comprises an open reading frame which encodes a Nef protein containing a tPA leader sequence fused to amino acid residue 6-216 of HIV-1 Nef (jfrl), wherein Leu-174 and Leu-175 are substituted with Ala-174 and Ala-175 (Ala-195 and Ala-196 in this tPA-based fusion protein). The nucleotide

sequence comprising the open reading frame of opt tpanef (LLAA) is disclosed herein as SEO ID NO:15, as shown below:

CATGGATGCA ATGAAGAGA GECTCTGCTG TGTGCTGCTG CTGTGTGGAG CAGTCTTCGT
TTCGCCCAGC GAGATCTCCT CCAAGAGGTC CGTGCCCGGC TGGTCCACCG TGAGGGAGAG

5 GATGAGGAGG GCCGAGCCCG CCGCCGACAG GGTGAGGAGG ACCGAGCCCG CCGCCGTGGG
CGTGGGCGCC GTGTCCAGGG ACCTGGAGAA GCACGGCGCC ATCACCTCCT CCAACACCGC
CGCCACCAAC GCCGACTGCG CCTGGCTGGA GGCCCAGGAG GACGAGGAGG TGGGCTTCCC
CGTGAGGCCC CAGGTGCCCC TGAGGCCCAT GACCTACAAG GGCGCCGTGG ACCTGTCCCA
CTTCCTGAAG GAGAAGGGCG GCCTGGAGGG CCTGATCCAC TCCCAGAAGA GGCAGGACAT

10 CCTGGACCTG TGGGTGTACC ACACCCAGGG CTACTTCCCC GACTGGCAGA ACTACACCCC
CGGCCCCGGC ATCAGGTTCC CCCTGACCTT CGGCTGGTG TTCAAGCTGG TGCCCGTGGA
GCCCGAGAAG GTGGAGGAGG CCAACGAGGG CGAGAACAAC TGCGCCGCCC ACCCCATGTC
CCAGCACGGC ATCGAGGACC CCGAGAAGGA GGTGCTGGAG TGGAGGTTCG ACTCCAAGCT
GGCCTTCCAC CACGTGGCCA GGGAGCTGCA CCCCGAGTAC TACAAGGACT GCTAAAGCCC

15 (SEQ ID NO:15).

The open reading frame of SEQ ID NO:7 encoding tPA-Nef (LLAA), disclosed herein as SEQ ID NO:16, is as follows:

Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Cys Gly Ala Val Phe Val Ser Pro Ser Glu Ile Ser Ser Lys Arg Ser Val Pro 20 Gly Trp Ser Thr Val Arg Glu Arg Met Arg Arg Ala Glu Pro Ala Ala Asp Arg Val Arg Arg Thr Glu Pro Ala Ala Val Gly Val Gly Ala Val Ser Arg Asp Leu Glu Lys His Gly Ala Ile Thr Ser Ser Asn Thr Ala Ala Thr Asn Ala Asp Cys Ala Trp Leu Glu Ala Gln Glu Asp Glu Glu Val Gly Phe Pro Val Arg Pro Gln Val Pro Leu Arg Pro Met Thr Tyr 25 Lys Gly Ala Val Asp Leu Ser His Phe Leu Lys Glu Lys Gly Leu Glu Gly Leu Ile His Ser Gln Lys Arg Gln Asp Ile Leu Asp Leu Trp Val Tyr His Thr Gln Gly Tyr Phe Pro Asp Trp Gln Asn Tyr Thr Pro Gly Pro Gly Ile Arg Phe Pro Leu Thr Phe Gly Trp Cys Phe Lys Leu Val Pro Val Glu Pro Glu Lys Val Glu Glu Ala Asn Glu Gly Glu Asn Asn Cys Ala Ala His Pro Met Ser Gln His Gly Ile Glu Asp Pro Glu 30 Lys Glu Val Leu Glu Trp Arg Phe Asp Ser Lys Leu Ala Phe His His Val Ala Arg Glu Leu His Pro Glu Tyr Tyr Lys Asp Cys (SEQ ID NO:16). An adenoviral vector of the present invention may comprise a DNA sequence, regardless of codon usage, which expresses a wild type or modified Nef protein as described herein, including but not limited to modified Nef proteins which comprise a 35 deletion or substitution of Gly 2, a deletion of substitution of Leu 174 and Leu 175

and/or inclusion of a leader sequence. Therefore, partial or fully codon optimized DNA vaccine expression vector constructs are preferred since such constructs should result in increased host expression. However, it is within the scope of the present invention to utilize "non-codon optimized" versions of the constructs disclosed herein, especially modified versions of HIV Nef which are shown to promote a substantial cellular immune response subsequent to host administration.

Figure 20A-C show nucleotide sequences at junctions between nef coding sequence and plasmid backbone of nef expression vectors V1Jns/nef (Figure 20A), V1Jns/nef(G2A,LLAA) (Figure 20B), V1Jns/tpanef (Figure 20C) and V1Jns/tpanef(LLAA) (Figure 20C, also). 5' and 3' flanking sequences of codon optimized nef or codon optimized nef mutant genes are indicated by bold/italic letters; nef and nef mutant coding sequences are indicated by plain letters. Also indicated (as underlined) are the restriction endonuclease sites involved in construction of respective nef expression vectors. V1Jns/tpanef and V1Jns/tpanef(LLAA) have identical sequences at the junctions.

Figure 21 shows a schematic presentation of nef and nef derivatives. Amino acid residues involved in Nef derivatives are presented. Glycine 2 and Leucine 174 and 175 are the sites involved in myristylation and dileucine motif, respectively.

20

25

30

35

5

10

15

EXAMPLE 19

MRKAd5Pol Construction and Virus Rescue

Steps performed in the construction of the vectors, including the pre-adenovirus plasmid - Key steps performed in the construction of the vectors, including the pre-adenovirus plasmid denoted MRKAd5pol, is depicted in Figure 22. Briefly, the adenoviral shuttle vector for the full-length inactivated HIV-1 pol gene is as follows. The vector MRKpdelE1(Pac/pIX/pack450)+CMVmin+BGHpA(str.) is a derivative of the shuttle vector used in the construction of the MRKAd5gag adenoviral pre-plasmid. The vector contains an expression cassette with the hCMV promoter (no intronA) and the bovine growth hormone polyadenylation signal. The expression unit has been inserted into the shuttle vector such that insertion of the gene of choice at a unique BgIII site will ensure the direction of transcription of the transgene will be Ad5 E1 parallel when inserted into the MRKpAd5(E1-/E3+)Cla1 (or MRKpAdHVE3) preplasmid. The vector, similar to the original shuttle vector contains the Pac1 site, extension to the packaging signal region, and extension to the pIX gene. The synthetic full-length codon-optimized HIV-1 pol gene was isolated directly from the plasmid pV1Ins-HIV-pol-inact(opt). Digestion of this plasmid with BgI II releases the pol

gene intact (comprising a codon optimized IA pol sequence as disclosed in SEQ ID NO:3). The pol fragment was gel purified and ligated into the MRKpdelE1(Pac/pIX/pack450)+CMVmin+BGHpA(str.) shuttle vector at the BgIII site. The clones were checked for the correct orientation of the gene by using restriction enzymes DraIII/Not1. A positive clone was isolated and named MRKpdel+hCMVmin+FL-pol+bGHpA(s). The genetic structure of this plasmid was verified by PCR, restriction enzyme and DNA sequencing. The pre-adenovirus plasmid was constructed as follows. Shuttle plasmid MRKpdel+hCMVmin+FLpol+bGHpA(S) was digested with restriction enzymes Pac1 and Bst1107 I (or its isoschizomer, BstZ107 I) and then co-transformed into E. coli strain BJ5183 with linearized (Cla1 digested) adenoviral backbone plasmid, MRKpAd(E1-/E3+)Cla1. The resulting pre-plasmid originally named MRKpAd+hCMVmin+FLpol+bGHpA(S)E3+ is now referred to as "pMRKAd5pol". The genetic structure of the resulting pMRKAd5pol was verified by PCR, restriction enzyme and DNA sequence analysis. The vectors were transformed into competent E. coli XL-1 Blue for preparative production. The recovered plasmid was verified by restriction enzyme digestion and DNA sequence analysis, and by expression of the pol transgene in transient transfection cell culture. The complete nucleotide sequence of this pMRKAd5HIV-1pol adenoviral vector is shown in Figure 26 A-AO.

5

10

15

20

25

30

35

Generation of research-grade recombinant adenovirus - The pre-adenovirus plasmid, pMRKAd5pol, was rescued as infectious virions in PER.C6® adherent monolayer cell culture. To rescue infectious virus, 12 μ g of pMRKAd5pol was digested with restriction enzyme Pacl (New England Biolabs) and 3.3 μ g was transfected per 6 cm dish of PER.C6® cells using the calcium phosphate coprecipitation technique (Cell Phect Transfection Kit, Amersham Pharmacia Biotech Inc.). Pacl digestion releases the viral genome from plasmid sequences allowing viral replication to occur after entry into PER.C6® cells. Infected cells and media were harvested 6-10 days post-transfection, after complete viral cytopathic effect (CPE) was observed. Infected cells and media were stored at \leq -60°C. This pol containing recombinant adenovirus is referred to herein as "MRKAd5pol". This recombinant adenovirus expresses an inactivated HIV-1 Pol protein as shown in SEQ ID NO:6.

EXAMPLE 20

MRKAd5Nef Construction and Virus Rescue

Construction of vector: shuttle plasmid and pre-adenovirus plasmid - Key steps performed in the construction of the vectors, including the pre-adenovirus

plasmid denoted MRKAd5nef, is depicted in Figure 23. Briefly, as shown in Example 19 above, the vector

5

10

15

20

25

30

35

MRKpdelE1(Pac/pIX/pack450)+CMVmin+BGHpA(str.) is the shuttle vector used in the construction of the MRKAd5gag adenoviral pre-plasmid. It has been modified to contain the *Pac*1 site, extension to the packaging signal region, and extension to the pIX gene. It contains an expression cassette with the hCMV promoter (no intronA) and the bovine growth hormone polyadenylation signal. The expression unit has been inserted into the shuttle vector such that insertion of the gene of choice at a unique *Bgl*11 site will ensure the direction of transcription of the transgene will be Ad5 E1 parallel when inserted into the MRKpAd5(E1-/E3+)Cla1 pre-plasmid. The synthetic full-length codon-optimized HIV-1 nef gene was isolated directly from the plasmid pV1Jns/nef (G2A,LLAA). Digestion of this plasmid with *Bgl*11 releases the pol gene intact, which comprises the nucleotide sequence as disclosed in SEQ ID NO:13. The nef fragment was gel purified and ligated into the

MRKpdelE1+CMVmin+BGHpA(str.) shuttle vector at the Bgl11 site. The clones were checked for correction orientation of the gene by using restriction enzyme Scal. A positive clone was isolated and named MRKpdelE1hCMVminFL-nefBGHpA(s). The genetic structure of this plasmid was verified by PCR, restriction enzyme and DNA sequencing. The pre-adenovirus plasmid was constructed as follows. Shuttle plasmid MRKpdelE1hCMVminFL-nefBGHpA(s) was digested with restriction enzymes Pac1 and Bst1107 I (or its isoschizomer, BstZ107 I) and then co-transformed into E. coli strain BJ5183 with linearized (Cla1 digested) adenoviral backbone plasmid, MRKpAd(E1/E3+)Cla1. The resulting pre-plasmid originally named MRKpdelE1hCMVminFL-nefBGHpA(s) is now referred to as "pMRKAd5nef". The genetic structure of the resulting pMRKAd5nef was verified by PCR, restriction enzyme and DNA sequence analysis. The vectors were transformed into competent E. coli XL-1 Blue for preparative production. The recovered plasmid was verified by restriction enzyme digestion and DNA sequence analysis, and by expression of the nef transgene in transient transfection cell culture. The complete nucleotide sequence of this pMRKAd5HIV-1nef adenoviral vector is shown in Figure 27A-AM.

Generation of research-grade recombinant adenovirus - The pre-adenovirus plasmid, pMRKAd5nef, was rescued as infectious virions in PER.C6[®] adherent monolayer cell culture. To rescue infectious virus, 12 μg of pMRKAdnef was digested with restriction enzyme Pac1 (New England Biolabs) and 3.3 μg was transfected per 6 cm dish of PER.C6[®] cells using the calcium phosphate coprecipitation technique (Cell Phect Transfection Kit, Amersham Pharmacia Biotech

Inc.). Pac1 digestion releases the viral genome from plasmid sequences allowing viral replication to occur after entry into PER.C6®cells. Infected cells and media were harvested 6-10 days post-transfection, after complete viral cytopathic effect (CPE) was observed. Infected cells and media were stored at \leq -60°C. This nef containing recombinant adenovirus is now referred to as "MRKAd5nef".

EXAMPLE 21

5

10

15

20

25

30

35

Construction of Murine CMV Promoter Containing Shuttle Vectors for Inactivated Pol and Nef/G2A,LLAA

The murine CMV (mCMV) was amplified from the plasmid pMH4 (supplied by Frank Graham, McMaster University) using the primer set: mCMV (Not I) Forward: 5'-ATA AGA ATG CGG CCG CCA TAT ACT GAG TCA TTA GG-3' (SEQ ID NO: 20); mCMV (Bgl II)Reverse: 5'-AAG GAA GAT CTA CCG ACG CTG GTC GCG CCT C-3' (SEQ ID NO:21). The underlined nucleotides represent the Not I and the $Bgl \Pi$ sites respectively for each primer. This PCR amplicon was used for the construction of the mCMV shuttle vector containing the transgene in the E1 parallel orientation. The hCMV promoter was removed from the original shuttle vector (containing the hCMV-gag-bGHpA transgene in the E1 parallel orientation) by digestion with Not I and Bgl II. The mCMV promoter (Not I/Bgl II digested PCR product) was inserted into the shuttle vector in a directional manner. The shuttle vector was then digested with $Bgl \ \Pi$ and the gag reporter gene ($Bgl \ \Pi$ fragment) was re-inserted back into the shuttle vector. Several clones were screened for correct orientation of the reporter gene. For the construction of the mCMV-gag in the E1 antiparallel orientation, the mCMV promoter was amplified from the plasmid pMH4 using the following primer set: mCMV (Asc I) Forward: 5'- ATA AGA ATG GCG CGC CAT ATA CTG AGT CAT TAG G (SEQ ID NO:22); mCMV (Bgl II) Reverse: 5' AAG GAA GAT CTA CCG ACG CTG GTC GCG CCT C (SEQ ID NO:23). The underlined nucleotides represent the Asc I and Bgl II sites, respectively for each primer. The shuttle vector containing the hCMV-gag transgene in the E1 antiparallel orientation was digested with Asc1 and Bgl11 to remove the hCMV-gag portion of the transgene. The mCMV promoter (Asc1/Bgl11 digested PCR product) was inserted into the shuttle vector in a directional manner. The vector was then digested with Bgl11 and the gag reporter gene (Bgl11 fragment) was re-inserted. Several clones were screened for correct orientation of the reporter gene. For each of the full length IA pol and full length nef/G2A,LLAA genes, cloning was performed using the unique

 $Bgl \ \Pi$ site within the mCMV-bGHpA shuttle vector. The pol and nef genes were excised from their respective pV1Jns plasmids by $Bgl \ \Pi$ digestion.

5

10

15

35

EXAMPLE 22

Construction of mCMV Full Length Inactivated Pol and Full Length nef/G2A.LLAA Adenovectors

Each of these transgenes of Example 21 were inserted into the modified shuttle vector in both the E1 parallel and E1 anti-parallel orientations. Pac1 and BstZ110I digestion of each shuttle vector was performed and each specific transgene fragment containing the flanking Ad5 sequences was isolated and co-transformed with Cla I digested MRKpAd5(E3+) or MRKpAd5(E3-) adenovector plasmids via bacterial homologous recombination in BJ5183 E. coli cells. Recombinant preplasmid adenovectors containing the various transgenes in both the E3- and E3+ versions (and in the E1 parallel and E1 antiparallel orientations) were subsequently prepared in large scale following transformation into XL-1 Blue E. coli cells and analyzed by restriction analysis and sequencing.

EXAMPLE 23

Construction of hCMV-tpa-nef (LLAA) Adenovector

The tpa-nef gene was amplified out from GMP grade pV1Jns-tpanef (LLAA) vector using the primer sets: Tpanef (BamHI) F 5'-ATT GGA TCC ATG GAT GCA ATG AAG AGA GGG (SEQ ID 24); Tpanef (BamHI) R 5'-ATA GGA TCC TTA GCA GTC CTT GTA GTA CTC G (SEQ ID NO:25). The resulting PCR product was digested with BamHI, gel purified and cloned into the Bgl II site of MRKAd5CMV-bGHpA shuttle vector (Bgl II digested and calf intestinal phosphatase treated). Clones containing the tpanef (LLAA) gene (see SEQ ID NO:15 for complet coding region) in the correct orientation with respect to the hCMV promoter were selected following Sca I digestion. The resulting MRKAd5tpanef shuttle vector was digested with Pac I and Bst Z1101 and cloned into the E3+MRKAd5 adenovector via bacterial homologous recombination techniques.

EXAMPLE 24

Immunogenicity of MRKAd5pol and MRKAd5nef Vaccine

Materials and Methods - Rodent Immunization - Groups of N=10 BALB/c

mice were immunized i.m. with the following vectors: (1) MRKAd5hCMV-IApol

(E3+) at either 10^7 vp and 10^9 vp; and (2) MRKAd5hCMV-IApol (E3-) at either

10^7 vp and 10^9 vp. At 7 weeks post dose, 5 of the 10 mice per cohort were boosted with the same vector and dose they initially received. At 3 weeks post the second does, sera and spleens were collected from all the animals for RT ELISA and IFNg ELIspot analyses, respectively. For all rodent immunizations, the Ad5 vectors were diluted in 5 mM Tris, 5% sucrose, 75 mM NaCl, 1 mM MgCl2, 0.005% polysorbate 80, pH 8.0. The total dose was injected to both quadricep muscles in 50 µL aliquots using a 0.3-mL insulin syringe with 28-1/2G needles (Becton-Dickinson, Franklin Lakes, NJ).

Groups of N=10 C57/BL6 mice were immunized i.m. with the following vectors: (1) MRKAd5hCMV-nef(G2A,LLAA) (E3+) at either 10^7 vp and 10^9 vp; (2) MRKAd5mCMV-nef(G2A,LLAA) (E3+) at either 10^7 vp and 10^9 vp; and (3) MRKAd5mCMV-tpanef(LLAA) (E3+) at either 10^7 vp and 10^9 vp. At 7 weeks post dose, 5 of the 10 mice per cohort were boosted with the same vector and dose they initially received. At 3 weeks post the second does, sera and spleens were collected from all the animals for RT ELISA and IFNg ELIspot analyses, respectively.

10

15

20

25

30

35

Non-human Primate immunization - Cohorts of 3 rhesus macaques (2-3 kg) were vaccinated with the following Ad vectors: (1) MRKAd5hCMV-IApol (E3+) at either 10^9 vp and 10^11 vp dose; and (2) MRKAd5hCMV-IApol (E3-) at either 10^9 vp and 10^11 vp; (3) MRKAd5hCMV-nef(G2A,LLAA) (E3+) at either 10^9 vp and 10^11 vp; and (4) MRKAd5mCMV-nef(G2A,LLAA) (E3+) at either 10^9 vp and 10^11 vp. The vaccine was administered to chemically restrained monkeys (10 mg/kg ketamine) by needle injection of two 0.5 mL aliquots of the Ad vectors (in 5 mM Tris, 5% sucrose, 75 mM NaCl, 1 mM MgCl₂, 0.005% polysorbate 80, pH 8.0) into both deltoid muscles. The animals were immunized twice at a 4 week interval (T=0, 4 weeks).

Murine anti-RT and anti-nef ELISA - Anti-RT titers were obtained following standard secondary antibody-based ELISA. Maxisorp plates (NUNC, Rochester; NY) were coated by overnight incubation with 100 μL of 1 μg/mL HIV-1 RT protein (Advanced Biotechnologies, Columbia, MD) in PBS. For anti-nef ELISA, 100 uL of 1 ug/mL HIV-1 nef (Advanced Biotechnologies, Columbia, MD) was used to coat the plates. The plates were washed with PBS/0.05% Tween 20 using Titertek MAP instrument (Hunstville, AL) and incubated for 2 h with 200 μL/well of blocking solution (PBS/0.05% tween/1% BSA). An initial serum dilution of 100-fold was performed followed by 4-fold serial dilution. 100-μL aliquots of serially diluted samples were added per well and incubated for 2 h at room temperature. The plates

were washed and 100 μ L of 1/1000-diluted HRP-rabbit anti-mouse IgG (ZYMED, San Francisco, CA) were added with 1 h incubation. The plates were washed thoroughly and soaked with 100 μ L 1,2-phenylenediamine dihydrochloride/hydrogen peroxide (DAKO, Norway) solution for 15 min. The reaction was quenched by adding 100 μ L of 0.5M H₂SO4 per well. OD₄₉₂ readings were recorded using Titertek Multiskan MCC/340 with S20 stacker. Endpoint titers were defined as the highest serum dilution that resulted in an absorbance value of greater than or equal to 0.1 OD₄₉₂ (2.5 times the background value).

-10

15

20

25

30

35

Non-human primate and murine ELIspot assays - The enzyme-linked immuno-spot (ELISpot) assay was utilized to enumerate antigen-specific INFγ-secreting cells from mouse spleens (Miyahira, et al. 1995, J. Immunol. Methods 181:45-54) or macaque PBMCs. Mouse spleens were pooled from 5 mice/cohort and single cell suspensions were prepared at 5x10⁶/mL in complete RPMI media (RPMI1640, 10% FBS, 2mM L-glutamine, 100U/mL Penicillin, 100 u/mL streptomycin, 10 mM Hepes, 50 uM β-ME). Rhesus PBMCs were prepared from 8-15 mL of heparinized blood following standard Ficoll gradient separation (Coligan, et al, 1998, Current Protocols in Immunology. John Wiley & Sons, Inc.). Multiscreen opaque plates (Millipore, France) were coated with 100 μL/well of either 5 μg/mL purified rat anti-mouse IFN-γ IgG1, clone R4-6A2 (Pharmingen, San Diego, CA), or 15 ug/mL mouse anti-human IFN-γ IgG_{2a} (Cat. No. 1598-00, R&D Systems, Minneapolis, MN) in PBS at 4°C overnight for murine or monkey assays, respectively. The plates were washed with PBS/penicillin/streptomycin and blocked with 200 μL/well of complete RPMI media for 37 °C for at least 2 h.

To each well, 50 μL of cell samples (4-5x10⁵ cells per well) and 50 μL of the antigen solution were added. To the control well, 50 μL of the media containing DMSO were added; for specific responses, either selected peptides or peptide pools (4 ug/mL per peptide final concentration) were added. For BALB/c mice immunized with the pol constructs, stimulation was conducted using a pool of CD4⁺-epitope containing 20-mer peptides (aa21-40, aa411-430, aa641-660, aa731-750, aa771-790) or a pool of CD8⁺-epitope containing peptides (aa201-220, aa311-330, aa781-800). For C57/BL6 mice immunized with the nef construct, either aa51-70 (CD8⁺ T cell epitope) or aa81-100 (CD4⁺) peptide derived from the nef sequence was added for specific stimulation. In monkeys, the responses against pol were evaluated using two pools (L and R) of 20-aa peptides that encompass the entire pol sequence and overlap by 10 amino acids. In monkeys vaccinated with the nef constructs, a single pool containing 20-mer peptides covering the entire HIV-1 nef sequence and overlapping

by 10 aa was used. Each sample/antigen mixture was performed in triplicate wells for murine samples or in duplicate wells for rhesus PBMCs. Plates were incubated at 37°C, 5% CO₂, 90% humidity for 20-24 h. The plates were washed with PBS/0.05% Tween 20 and incubated with 100 μL/well of either 1.25 μg/mL biotin-conjugated rat anti-mouse IFN-γ mAb, clone XMG1.2 (Pharmingen) or of 0.1 ug/mL biotinylated anti-human IFN-gamma goat polyclonal antibody (R&D Systems) at 4°C overnight. The plates were washed and incubated with 100 μL/well 1/2500 dilution of strepavidin-alkaline phosphatase conjugate (Pharmingen) in PBS/0.005% Tween/5% FBS for 30 min at 37 °C. Spots were developed by incubating with 100 μL/well 1-step NBT/BCIP (Pierce Chemicals) for 6-10 min. The plates were washed with water and allowed to air dry. The number of spots in each well was determined using a dissecting microscope and the data normalized to 10⁶ cell input.

Non-human Primate anti-RT ELISA - The pol-specific antibodies in the monkeys were measured in a competitive RT EIA assay, wherein sample activity is determined by the ability to block RT antigen from binding to coating antibody on the plate well. Briefly, Maxisorp plates were coated with saturating amounts of pol positive human serum (#97111234). 250 uL of each sample is incubated with 15 uL of 266 ng/mL RT recombinant protein (in RCM 563, 1% BSA, 0.1% tween, 0.1% NaN₃) and 20 uL of lysis buffer (Coulter p24 antigen assay kit) for 15 min at room temperature. Similar mixtures are prepared using serially diluted samples of a standard and a negative control which defines maximum RT binding. 200 uL/well of each sample and standard were added to the washed plate and the plate incubated 16-24 h at room temperature. Bound RT is quantified following the procedures described in Coulter p24 assay kit and reported in milliMerck units per mL arbitrarily defined by the chosen standard.

Results - Rodent Studies - BALB/c mice (n=5 mice/cohort) were immunized once or twice with varying doses of MRKAd5hCMV-IApol(E3+) and MRKAd5hCMV-IApol(E3-). At 3 weeks after the second dose, Anti-pol IgG levels were determined by an ELISA assay using RT as a surrogate antigen. Cellular response were quantified via IFNγ ELISpot assay against pools of pol-epitope containing peptides. The results of these assays are summarized in Table 10. The results indicate that the mouse vaccinees exhibited detectable anti-RT IgGs with an adenovector dose as low as 10⁴7 vp. The humoral responses are highly dosedependent and are boostable with a second immunization. One or two doses of either pol vectors elicit high frequencies of antigen-specific CD4⁺ and CD8⁺ T cells; the responses are weakly dose-dependent but are boostable with a second immunization.

Table 10. Immunogenicity of MRKAd5pol Vectors in BALB/c mice.

				An	ti-RT IgG Tite	rs*	S	FC/10^6 cell	s*
Group	Vaccine	Dose	No. of Doses	GMT	+SE	-SE	Medlum	CD4+ peptide pool	CD8+ peptide pool
1	MRKAd5hCMVFLpol (E3+)	10^7 vp	2 1	310419 919	301785 372	153020 265	1(1) 1(1)	75(4) 72(9)	2313(67) 533(41)
2	MRKAdShCMVFLpol (E3+)	10^9 vp	2 1	1638400 ⁶ 713155	0 528520	0 303555	2(2) 1(1)	114(9) 48(7)	2063(182) 733(89)
3	MRKAd5hCMVFLpol (E3-)	10^7 vp	2	310419 6400	385218 14013	172097 4393	Q(0) 10(8)	223(7) 141(21)	2607(27) 409(28)
4	MRKAd5hCMVFLpol (E3-)	10^9 vp	2	1838400 ^b 1241675 ^b	0 396725	0 300661	1(1) 0(0)	160(13) 39(13)	2385(11) 833(83)
5	Naive	none	none	57	9	7	9(2)	11(4)	10(1)

^{*}GMT, geometric mean titer of the cohort of 5 mice; SE, standard error of the gemetric mean

5 C57/BL6 mice were immunized once or twice with varying doses of MRKAd5hCMV-nef(G2A,LLAA) (E3+), MRKAd5mCMV-nef(G2A,LLAA) (E3+) at either 10^7 vp and(3) MRKAd5mCMV-tpanef(LLAA) (E3+) at either 10^7 vp and 10^9 vp. The immune response were analyzed using similar protocols and the results are listed in Table 11. While anti-nef IgG responses could not be detected in this model system with any of the constructs, there are strong indications of a cellular immunity generated against nef using the ELIspot assay.

Table 11. Immunogenicity of MRKAd5nef Vectors in C57/BL6 mice.

				Ar	ti-nef lgG Tit	ere"	8	FC/10^6 cell	3,
Group	Vaccine	Dose	No. of Doses	GMT	+SE	-SE	Medium	Ba51-70 CD8+	aa81-100 CD4+
1	MRKAd5hCMVFLnef (E3+)	10^7 vp	1	174 132	70 42	50 32	1(1) 0(0)	23(1) 0(0)	1(1) 0(0)
2	MRKAd5hCMVFLnef (ES+)	10^9 vp	2 1	174 132	70 42	50 32	0(0) 1(1)	61(7) 62(7)	4(2) 3(1)
3	MRKAd5mCMVFLnet (E3+)	10^7 vp	2	132 115	42 46	32 33	3(1) 3(2)	15(5) 3(2)	5(2) 4(2)
4	MRKAd5mCMVFLnef (E3+)	10 ^9 vp	2	132 132	42 42	32 32	4(2) 2(1)	83(13) 29(2)	5(1) 4(0)
5	MRKAd5mCMVtpanel(E3+)	10^7 vp	2	132 100	42 0	32 0	3(2) 3(1)	14(2) 13(4)	5(1) 10(3)
6	MRKAd5mCMVtpanel(E3+)	10^9 vp	2	230 115	170 46	98 33	3(2) 7(1)	145(29) 151(14)	4(0) 10(0)
-,	Naîve	none	none	152	78	52 -	21(2)	· 18(6)	26(3)

^{*}GMT, geometric mean titer of the cohort of 5 mice; SE, standard error of the gemetric mean

15

Monkey Studies - Cohorts of 3 rhesus macaques were immunized with 2 doses of MRKAd5hCMV-IApol(E3+) and MRKAd5hCMV-IApol(E3-). The number of antigen-specific T cells (per million PBMCs) were enumerated using one of two

Near or at the upper limit of the serial dilution; hence, could be greater than this value

[&]quot;No. of Spot-forming Cells per million spiecnoytes; mean values of triplicates are reported along with standard errors in parenthesis.

No. of spot-forming cells per million splecnoyles; mean values of triplicates are reported along with standard errors in parentheals.

peptide pools (L and R) that cover the entire pol sequence; the results are listed in Table 12. Moderate-to-strong T cell responses were detected in the vaccinees using either constructs even at a low dose of 10^9 vp. Longitudinal analyses of the anti-RT antibody titers in the animals suggest that the pol transgene product is expressed efficiently to elicit a humoral response (Table 13). It would appear that generally higher immune responses were observed in animals that received the E3- construct compared to the E3+ virus.

Table 12. Pol-specific T Cell Responses in MRKAd5pol Immunized Rhesus

10	Macaques.
----	-----------

Vaccine (T=0,4 wks)	Monk #		Prebleed			T=4			T=7			Ta16	
Vaccina (140) 4 mm/	titotta «	Mock	Pol L	Pal R	Mock	Pol L	Pol R	Mock	Pol L	Pol R	Mock	Pol L	Pol R
MRKACENCIMV-IApal(E3+)	99C100	1	0	D	1	38	31	0	52	148	0	49	715
10^11 vo	99C215	i	2	2	10	98	249	1	109	305	22	88	250
10-11-VP	99D2D1	5	5	4	6	149	85	0	40	35	0	35	18
MRKAd5hCMV-IApol(E3+)	99D212	0	2	0	4	331	114	0	58	14	0	6	6
10/9 VP	990180	٥	4	2	0	19	192	4	36	156	5	38	108
10,745	99C201	8	5	21	6	62	62	0	18	32	١ ١	14	65
MRK ActinCMV-I Apol(E3-)	99D239	5	2	2	20	82	172	1	66	114	7	21	40
1041 VD	99C186	4	12	6	5	120	421	2	271	489	16	875	530
	99C084	1	8	9	8	84	464	0	14	238	1	24	264
MRKAd5hCMV-IAcci(E3-)	007C	10	10	8	12	724	745	4	322	376	4	188	178
10/9 VD	CDIG	{ 2	0	1	5	474	468	0	232	212	0	101	121
, с. тр	0011	В	6	12	10	98	110	5	60	80	8	25	34
Nave	083Q	nd	nd	nd	nd	nd	nd	4	2	2	2	1	2

nd, not determined Reported are SFC per million PENCs; mean of duplicate wells.

Table 13. Anti-RT Ig Levels in MRKAd5pol Immunized macaques.

Vaccine/Monkey Tag	T=4	T =7	T=12	T=16
MRKAd5hCMV-IApol(E3+), 10^11 vp				
99C100	61	1999	5928	4768
99C215	81	1541	2356	2767
99D201	53	336	539	387
MRKAd5hCMV-IApol(E3+), 10^9 vp				
99D212	10	40	49	68
99D180	<10	36	79	93
99C201	<10	37	71	76
MRKAd5hCMV-IApol(E3-), 10^11 vp	 			
99D239	44	460	1234	1015
99C186	21	· 233 ·	480	345
990084	235	2637	2858	1626
MRKAd5hCMV-IApol(E3-), 10^9 vp				
CC7C	32	175	306	235
Φ16	20	140	273	419
Φ11	15	112	149	237

When rhesus macaques were immunized i.m. with two doses of MRKAd5nef constructs, vigorous T cell responses ranging from 100 to as high as 1100 per million were observed in 8 of 12 vaccinees (Table 14). The efficacies of the mCMV- and hCMV- driven nef constructs are comparable on the basis of the data generated thus far.

5

10

15

20

25

Table 14. Nef-specific T cell Responses in MRKAd5nef Immunized Rhesus

Vaccine (T=0,4 wks)	Monk #	Pi	18	To	=4	Ţ	- 7	T	16
		Mock	Nef	Mock	Nef	Mock	Nef	Mock	Nef
MRKAd5hCMV-nef(G2A,LLAA) (E3+)	CD2D	0	4	31	440	4	368	1	251
10^11 vp	CC7B	0 1	0	2	521	0	178	1	1522
·	CC61	2	9	31	112	0	108	11	100
MRKAd5hCMV-nef(G2A,LLAA) (E3+)	CC2K	8	9	6	52	0	35	0	15
10^9 vp	CD15	5	4	30	898	2	586	٥	434
·	CD16	6	1	6	1146	0	369	1	212
MRKAd5mCMV-nef(G2A,LLAA) (E3+)	99D191	1	- 6	4	814	0	298	2	419
10^11 vp	99D144	4	6	5	434	0	1100	2	932
·	99C193	1	2	1	58	1 1	22	0	64
MRKAd5mCMV-net(G2A,LLAA) (E3+)	99D224	1	11	14	231	1	125	0	70
10^9 vp	99D250	8	9	4	108	0	54	0	5
·	99C120	1 1	6	20	299	٥	92	0	79
Naïve	083Q	nd	nd	18	22	4	- 5	2	1

EXAMPLE 25

Comparison of Clade B vs. Clade C T Cell Responses in HIV-Infected Subjects
PBMC samples collected from two dozens of patients infected with HIV-1 in
US were tested in ELISPOT assays with peptide pools of 20-mer peptides overlapping
by 10 amino acids. Four different peptide pools were tested for cross-clade
recognition, and they were either derived from a clade B-based isolate (gag H-b; nefb) or a clade C-based isolate (gag H-c, nef-c). Data in Table 15 shows that T cells
from these patients presumably infected with clade B HIV-1 could recognize clade C
gag and nef antigens in ELISPOT assay. Correlation analysis further demonstrated
that these T cell responses against clade C gag peptide pool were about 60% of the
clade B counterpart (Figure 24), while the T cell responses against clade C nef were
about 85% of the clade B counterpart (Figure 25). These results suggest that cellular
immune responses generated in patients infected with clade B HIV-1 can recognize
gag and nef antigens derived from clade C HIV-1. These data show that a HIV
vaccine, such as a DNA or MRKAd5-based adenoviral vaccine expressing a clade B

gag and/or nef antigen will potentially have the ability to provide a prophylactic and/or therapetic advantage on a global scale.

Table 15
Responses Shown as the Number of gIFN-Secreting T Cells per Million PBMCs

subject	bleed date	gag epitope #	mock	gag H-b	gagH-c	nef-b	nef-c
		(from mapping)					
#100	19-Jul-99	12	10	3950	1385	1295	1300
#101	25-Jul-99	3	15	3885	1280	na	1020
#102	25-Jul-99	4	15	1740	850	1255	1785
#104	7-Jun-99	2	5	1355	1185	na	1060
#107	11-Oct-99	2	25	3305	2795	670	870
#405	11-Jul-99	2	15	4575	3180	1700	1500
#501	19-Jul-99	2	15	1100	570	3365	3460
#505	18-Jul-99	5	10	2145	1725	1235	na
#506	28-Feb-99	2	25	150	45	400	610
#701	28-Mar-99	5	30	7620	4775	3320	2780
#709	17-May-99	3	15	2785	1945	1090	1630
#710	24-May-99	4	5	1055	1080	2210	2140
·				1			

10

20

EXAMPLE 26 Characterization and Production of MRKAd5pol and MRKAd5nef Vectors in Roller Bottles

Expansion of nef and pol Adenovectors - Nef and pol CsCl purified MRKAd5 seeds were used to infect roller bottles to produce P4 virus to be used as a seed for further experiments. P4 MRKAd5 pol and nef vectors were used to infect roller bottles at an MOI 280 vp/cell, except for hCMV-tpa-nef [E3+] which was infected at an MOI of 125 due to low titers of seed obtained at P4.

Table 16 Viral particle concentrations for P5 nef and pol adenovectors

Adenovector	AEX Titer (10 ¹⁰ vp/ml culture)	AEX Titer (10 ⁴ vp/cell)	Amplification Ratio
hCMV-FL-nef [E3+]	1.1	0.9	30
mCMV-FL-nef [E3+]	2.2	2.1	75
hCMV-tpa-nef [E3+]	0.07	0.1	5
mCMV-tpa-nef [E3+]	1.3	0.9	35
hCMV-FL-pol [E3+]	2.7	2.1	75
hCMV-FL-pol [E3-]	1.9	1.3	45

5 Roller Bottle Passaging - Passaging of the pol and nef constructs continued through passage seven. Cell-associated (freeze/thaw lysis) and whole broth (tritonlysis) titers obtained in all passages were very consistent. In general, MRKAd5pol is ca. 70% as productive as MRKAd5gag while MRKAd5nef is ca. 25% as productive as MRKAd5gag. Samples of P7 virus for both constructs were analyzed by V&CB by restriction digest analysis and did not show any rearrangements.

Table 17. Passage Six Viral Productivity for MRKAd5pol and MRKAd5nef

			0° cells/ml), lity (%) Harvest	Cell Passage Number	AEX Titer (Cell Associated) 10 ¹⁰ vp/ml culture	Titzr 104 vp/ccil	Amplification Ratio	Triton Lysis Titer 10 ¹⁰ vp/ml culture
hCMV-FL-nef [B3+]	pool	1.22, 85%		62	0.8	0.7	25	1.6
]	1		0.99, 62%					
	2		1.10,72%	})		}	
bCMV-FL-pol (E3+)	pool	1.42, 89%		62	4.5	3.2	115	7.0
]	. 1		1.22, 70%					
	2		1.42, 74%	1]	

15 Table 18. Passage Seven Viral Productivity for MRKAd5pol and MRKAd5nef

15 14510		Xviable (1) Viabil Infection		Cell Passage Number	AEX Titer (Cell Associated) 10 ⁸⁰ vp/ml culture	Titer 10 ⁴ vp/cell	Amplification Ratio	Triton Lysis Titer 10 ^{to} vp/ml culture
hCMV-FL-acf (B3+)	Pool	1.33, 90%		66	1.0	0.8	29	2.1
\	- 1		0.96, 70%					
	2		1.18, 73%	.}	1			
bCMV-FL-pol [E3+]	Pool	0.90*, 90%		56	4.2	4.7	168	6.5
1	1		1.18, 88%					
Į.	2		1.04, 80%					

MRKAd5nef and MRKAd5pol Viral Production Kinetics - A timecourse experiment was carried out in roller bottles to determine if the viral production kinetics of the MRKAd5pol and MRKAd5nef vectors were similar to those of MRKAd5gag. PER.C6[®] cells in roller bottle cultures were infected at an MOI of 280 vp/cells with P5 MRKAd5pol, P5 MRKAd5nef and P7 MRKAd5gag; for each adenovector, two infected bottles were sampled at 24, 36, 48, and 60 hours post infection. In addition, two bottles were left unsampled until 48 hpi when they were harvested under the Phase I process conditions. The anion-exchange HPLC viral particle concentrations of the freeze-thaw recovered cell associated virus at the 24, 36,

48, and 60 hpi timepoints are shown in Figure 29A-B. The QPA titers show a similar trend (data not shown).

Comparison of hCMV- and mCMV-FL-nef - As the titers obtained with MRKAd5nef construct (hCMV-FL-nef) were lower than those obtained with MRKAd5gag or MRKAd5pol, a viral productivity comparison experiment was performed with mCMV-FL-nef. For each of the two adenovectors (hCMV- and mCMV-FL-nef), two roller bottles were infected at an MOI of 280 vp/cell with passage five clarified lysate. The macroscopic and microscopic observations of the four roller bottles were identical at the time of harvest. Analysis of the clarified lysate produced indicated a higher viral particle concentration in the bottles infected with mCMV-FL-nef, as shown in Table 19. It is stipulated that the higher productivity with mCMV promoter driven nef vector is due to lower nef expression levels in PHR.C6® cells- experiments are underway at V&CB to measure nef expression levels.

Table 19. Passage Six Viral Productivity Comparison of hCMV- and mCMV-FL-nef

	[Xv (10 ⁶ cells/m	l), Viability (%)	Cell Passage	AEX Titer	Titer	Amplification	Triton Lysis Titer
		Infection	Harvest	Number	10 ¹⁰ vp/ml culture	10° vp/cell	Ratio	10 ¹⁰ vp/ml culture
hCMV-FL-nef	Pool	1.11, 91%		60	1.5	1.4	50	2.8
(MRKAd5nef)	1		1.23,75%					
	2		1.34,74%		[
mCMV-FL-nef	Pool	1.11, 91%		60	2.3	2.1	75	4.6
	1		1.49, 84%					
	2		1.18, 77%					

20

25

30

5

10

EXAMPLE 27

Characterization and Large Scale Production of MRKAd5nef Virus in Bioreactors

Materials and Methods - The experiment of the present example was run twice under the following conditions: 36.5°C, DO 30%, pH 7.30, 150rpm agitation rate, no sparging, Life Technologies (Gibco, Invitrogen) 293 SFM II (with 6mM L-glutamine), 0.5M NaOH as base for pH control. During the first run (B20010115), two 10L stirred vessel bioreactors were inoculated with PER.C6® cells at a concentration of 0.2x106 cells/ml. Cells were grown until they reached a cell concentration of approximately 1x106 cells/ml. The cells were infected with uncloned MRKAd5nef (G2A,LLAA) at a MOI of 280 virus particles (vp)/cell. For the second batch (B20010202), the same procedure as the first run was used, except the cells

were infected with cloned MRAd5nef. During both runs, the bioreactors were harvested 48 hours post-infection. Samples were taken and virus concentrations were determined from whole broth (with triton lysis), supernatant, and cell pellets (3 X freeze/thaw) with the AEX and QPA assays. Metabolites were measured with BioProfile 250 throughout the process.

Table 20: Experimental Conditions

Temperature	36.5 ℃	
DO	30%	
PH	7.30	
Agitation	150 rpm	
Sparging	None	•

Table 21: Virus source used for experiments.

10

5

Run	Batch ID	Cloned/Uncloned MRKAd5nef	i MOI (vp/cells)		
#1	B20010115-1	Uncloned	280		
	B20010115-2	Uncloned	280		
#2	B20010202-1	Cloned	280		
	B20010202-2	Cloned	280		

Results - Table 22 and 23 show an the ability to scale up production of MRKAd5nef by growth in a bioreactor.

15

Table 22: Virus Concentration as measured by the AEX assay

Run	Batch ID	Cloned/Uncloned	Virus Concentration @ 48hpi (1x10 ¹³ vp/L)					
		MRKAd5nef	Supernatant	Clarified Lysate	Total	Triton Lysate		
#1	B20010115-1	Uncloned	0.72	3.26	3.98	5.76		
	B20010115-2	Uncloned	0.38	1.67	2.05	2.46		
#2	B20010202-1	Cloned	0.80	6.00	6.80	8.88_		
	B20010202-2	Cloned	0.50	6.00	6.50	8.47		

Table 23: Virus Titers as measured by the QPA assay

Run	Batch ID	Cloned/Uncloned	Virus Concentration @ 48hpi (1x1011 TU/L)					
		MRKAd5nef	Whole Broth	Supernatant	Clarified Lysate	Total	Triton Lysate	
#1	B20010115-1	Uncloned	0.13	1.12	1.76	2.88	11.28	
	B20010115-2	Uncloned	0.14	0.73	1.54	2.27	5.86	
#2	B20010202-1	Cloned	0.14	0.97	1.62	2.69	11.89	
	B20010202-2	Cloned	0.14	1.17	1.70	2.97	12.47	

20

The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art

from the foregoing description. Such modifications are intended to fall within the scope of the appended claims.

EXAMPLE 28

MRKAd5HIV-1gag Boosting of DNA-Primed Animals

5

10

15

20

25

30

Groups of 3-5 rhesus macaques were immunized with (a) 5 mgs of V1Jns-Flgag (pVIJnsCMV(no intron)-FL-gag-bGHpA), (b) 5 mgs of V1Jns-Flgag formulated with 45 mgs of a non-ionic block copolymer CRL1005, or (c) 5 mgs of V1Jns-Flgag formulated with 7.5 mgs of CRL1005 and 0.6 mM benzalkonium chloride at weeks 0, 4, and 8. All animals received a single dose of 10e7 viral particles (vp) of the MRKAd5HIV-1gag at week 26. Note: 10e7 is too low to prime or boost effectively when used as a single modality (dose is selected to mimic preexposure to adenovirus); see Figure 32.

Blood samples were collected from all animals at several time points and peripheral blood mononuclear cells (PBMCs) were prepared using standard Ficoll method. The PBMCs were counted and analyzed for gamma-interferon secretion using the ELISpot assay (Table 24). For each monkey, the PBMCs were incubated overnight either in the absence (medium) or presence of a pool (called "gag H") of 50 20-aa long peptides that encompass the entire HIV-1 gag sequence.

The results indicate that MRKAd5HIV-1gag was very effective in boosting the T cell immune responses in these monkeys. At week 28 or 2 weeks after the viral boost, the number of gag-specific T cells per million PBMCs increased 2-48 fold compared to the levels observed at week 24 or 2 weeks prior to the boost.

The PBMCs were also analyzed by intracellular gamma-interferon staining prior to (at week 10) and after the MRKAd5gag boost (at week 30). The results for select animals are shown on Figure 31. The results indicate that (a) immunization with DNA/adjuvant formulation elicited T cell responses which can either be balanced, CD4⁺-biased or CD8⁺-biased, and (b) boosting with the MRKAd5gag construct produced in all cases a strongly CD8⁺-biased response. These results suggest that boosting with MRKAd5HIV-1gag construct is able to improve the levels of antigen-specific CD8⁺ T cells.

☞ 은 ~ 959 1915 1549 1229 22822 **克克袋虫** 答名を答び 4 × 4 2 0 22 22 68 68 26228 S 5 5 5 5 2-00-18 to 2 to 8 2 4 2 85-85 8 4 8 ឧទ្ធទ •••₹2 | Table 24, Boosting of DNA/Adjuvant-Primed Ribesus Monkeys with MRKAd5gag
| Number of SFChmilton PBMC3 | Boost | Table | Monke | Table | Monke | Table | Medium | Table | Mediu AWZD CAAR CBSB CBSW CBSW CCTC CCTX AW3P AKBB MRIKAdSgag(E3+) 10v7 vp MP3CAdSgag(E3+) 10*7 vp CRL1005/7.5 mgs + 0.6 mM BAK DNA/6mgs + CRL 1005/45mgs A, not available

F 55 88

978 978 988 1831 1831

EXAMPLE 29

Construction of gagpol fusion for MRKAd5gagpol fusion constructs

The open reading frames for the codon-optimized HIV-1 gag gene was fused directly to the open reading frame of the IA pol gene (consisting of RT, RNAseH and integrase domains) by stepwise PCR. Because the gene (SEQ ID NO: 38) does not include the protease gene and the frameshift sequence, it encodes a single polypeptide of the combined size of p55, RT, RNAse H and integrase (1350 amino acids; SEQ ID NO: 39).

The fragment that extends from the BstEII site within the gag gene to the last non-stop codon was ligated via PCR to a fragment that extends from the start codon of the IApol to a unique BamHI site. This fragment was digested with BstEII and BamHI. Construction of gag-IApol fusion was achieved via three-fragment ligation involving the PstI-BstEII gag digestion fragment, the BstEII/BamHI digested PCR product and long PstI/BamHI V1R-FLpol backbone fragment.

10

15

20

30

35

The MRKAd5-gagpol adenovirus vector was constructed using the BglII fragment of the V1R-gagpol containing the entire ORF of gag-IApol fusion gene.

EXAMPLE 30

Immunogenicity Studies in Non-Human Primates

Cohorts of three (3) macaques were immunized with 10e8 or 10e10 viral particles (vp) of one of the following MRKAd5 HIV-1 vaccines: (1) MRKAd5gag; (2) MRKAd5pol; (3) MRKAd5nef; (4) a mixture containing equal amounts of MRKAd5gag, MRKAd5pol, and MRKAd5nef, or (5) a mixture of equal amounts of MRKAd5gagpol and MRKAd5nef. The vaccines were administered at weeks 0 and 4.

The T cell responses against each of the HIV-1 antigens were assayed by IFN-gamma ELISpot assay using pools of 20-aa peptides that encompass the entire protein sequence of each antigen. The results (Table 25) are expressed as the number of spot-forming cells (sfc) per million peripheral blood mononuclear cells (PBMC) that respond to each of the peptide pools.

Results indicate the following observations: (1) each of the single gene constructs (MRKAd5gag, MRKAd5pol, or MRKAd5nef) is able to elicit high levels of antigen-specific T cells in monkeys; (2) the single-gene MRKAd5 constructs can be mixed as a multi-cocktail formulation capable of eliciting very broad T cell responses against gag, pol, and nef; (3) the MRKAd5 vector expressing the fusion

protein of gag plus IA pol is capable of inducing strong T cell responses to both gag and pol.

Table 25. Evaluation of Mixtures of MRKAd5 vectors expressing humanized

HIV-1 gag, pol, gagpol, nef in rhesus macaques

10

Grp#	Vaccine	Monk#			T=6 wks		
	T=0, 4 wks	<u> </u>	Mock	Gag H	Pol - 1	Pol - 2	Nef
1	MRKAd5 gag	CB9V	0	15	-	-	-
·	10^10 vp	CD19	0 .	374	-	-	•
	·	109H	1	843	-	•	•
2	MRKAd5 gag	99D130	1	948	-	•	-
- 1	10^8 vp	W277	16	324	-	•	•
}		143H	4	595	-	-	•
3	MRKAd5 pol	CC1X	4	•	46	256	-
	10^10 vp	AW3W	3	-	463	550	-
	·	AV43	6	•	95	1333	•
4	MRKAd5 pol	AW38	1	-	19	30	-
1	10^8 vp	CC8K	0	-	50	995	-
		CC21	1	-	33	· 436	-
5	MRKAd5 nef	076Q	9	-	-	-	1204
	10^10 vp	091Q	4			١ ٠	85
		083Q	0	-	-	1 .	176
6	MRKAd5 nef	00C029	1		-	-	114
1	10^8 vp	98D022	6	-	-	-	170
		98D160	3	-	-	-	198
7	MRKAd5gag+MRKAd5pol+MRKAd5nef	99D251	3	206	15	193	120
	10^10 vp each	05H	3	135	21	9	638
		00C016	3	26	4	51	23
8	MRKAd5gag+MRKAd5pol+MRKAd5nef	99D215	1	171	18	193	240
ĺ	10^8 vp each	81H	5	73	6	14	243
		12H	8	1140	115	811	719
9	MRKAd5gagpol +MRKAd5 nef	99D211	0	83	56	838	725
	10^10 vp each	22H	4	385	119	1194	1915
		61H	4	343	11	765	853
10	MRKAd5gagpol +MRKAd5 nef	34H	3	78	19	5	75
l	10^8 vp each	48H	1 1	65	105	46	43
i		70H	5	158	15	220	191

Indicated are numbers of spot-forming cells per million PBMCS against the peptide pools. Mock, no peptides; gag H, fifty 20-aa peptides encompassing p55 sequence; pol-1, 20-aa peptides representing N-terminal half of IA pol; pol-2, 20-aa peptides representing the carboxy-terminal half of IA pol; nef, 20-aa peptides encompassing the entire wild-type nef sequence. Responses to the antigens prior to the first immunization did not exceed 40 sfc/10^6 PBMC.

WHAT IS CLAIMED IS

10

A recombinant adenoviral vaccine vector at least partially deleted in
 E1 and devoid of E1 activity, comprising:

- a) an adenovirus cis-acting packaging region corresponding to from about base pair 1 to between from about base pair 400 to about base pair 458 of a wildtype adenovirus genome; and
- b) a gene encoding an HIV protein or immunologically relevant modification thereof.
- A vector in accordance with claim 1 comprising a packaging region corresponding to from about base pair 1 to about base pair 450 of a wildtype adenovirus genome.
- 3. A vector in accordance with claim 1 further comprising nucleotides
 15 corresponding to between from about base pair 3511 to about 3524 to about base pair
 5798 of a wildtype adenovirus genome.
 - 4. A vector in accordance with claim 3 comprising base pairs corresponding to 1-450 and 3511-5798 of a wildtype adenovirus genome.
- 5. A vector in accordance with claim 4 which is deleted of base pairs 20 451-3510.
 - A vector in accordance with claim 1 which is at least partially deleted in E3.
 - 7. A vector in accordance with claim 6 wherein the E3 deleted region is from base pairs 28,133-30,818.

8. A vector in accordance with claim 1 wherein the gene encoding the HIV protein or modification thereof comprises codons optimized for expression in a human.

- 9. A vector in accordance with claim 1 wherein the vector comprises a gene expression cassette comprising:
 - a) a nucleic acid encoding a protein;

5

15

20

- b) a heterologous promoter operatively linked to the nucleic acid encoding the protein; and
 - (c) a transcription termination sequence.
- 10. A vector in accordance with claim 9 wherein the gene expression cassette is inserted into the E1 region.
 - 11. An adenoviral vector in accordance with claim 9 wherein the gene expression cassette is in an E1 parallel orientation
 - 12. An adenoviral vector in accordance with claim 9 wherein the gene expression cassette is in an B1 antiparallel orientation.
 - 13. An adenoviral vector in accordance with claim 9 wherein the promoter is a cytomegalovirus promoter devoid of intronic sequences.
 - 14. An adenoviral vector in accordance with claim 13 wherein the promoter is an immediate early human cytomegalovirus promoter.
 - 15. An adenoviral vector in accordance with claim 9 wherein the promoter is a murine cytomegalovirus promoter.
 - 16. An adenoviral vector in accordance with claim 9 wherein the transcription termination sequence is a bovine growth hormone polyadenylation and transcription termination sequence.

17. An adenoviral vector in accordance with claim 9 wherein the transcription termination sequence is a synthetic polyadenylation signal (SPA).

- 18. A cell comprising the adenoviral vector of claim 1.
- 19. Recombinant, replication-defective adenovirus particles harvested
 and purified subsequent to transfection of the adenoviral vector of claim 1 into a cell
 line which expresses adenovirus E1 protein at complementing levels.
 - 20. An HIV vaccine composition comprising purified adenovirus particles of claim 19.
- 21. An HIV vaccine composition of claim 20 which comprises aphysiologically acceptable carrier.
 - 22. A method of producing recombinant, replication defective adenovirus particles containing the adenoviral genome of the adenoviral vector of claim 1 which comprises introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and harvesting the resultant recombinant, replication-defective adenovirus.
 - 23. A method according to claim 22 wherein the cell is a PER.C6® cell.

15

- 24. A method of generating a cellular-mediated immune response against HIV in an individual comprising administering to the individual a vaccine of claim 21.
 - 25. A method according to claim 24 which further comprises administration to the individual a DNA plasmid vaccine, optionally administered with a biologically effective adjuvant, protein or other agent capable of increasing the immune response.

26. A method according to claim 25 wherein the DNA plasmid vaccine is administered to the individual prior to administration of an adenovirus vaccine.

- 27. A method according to claim 24 wherein the adenovirus vaccine is
 5 preceded by an adenovirus vaccine of a different serotype.
 - 28. A method according to claim 24 which comprises administering and readministering the adenovirus vaccine vector to the individual.
 - 29. An adenoviral vector in accordance with claim 1 wherein the HIV protein is HIV gag or an immunologically relevant modification thereof.
- 30. An adenoviral vector in accordance with claim 9 wherein the gene expression cassette comprises an open reading frame encoding an HIV gag protein or immunologically relevant modification thereof.
 - 31. A recombinant adenoviral vaccine vector at least partially deleted in E1 and devoid of E1 activity, comprising:
- a) an adenovirus cis-acting packaging region corresponding to from about base pair 1 to about base pair 450 and from about 3511 to about 5798 of a wildtype adenovirus genome, and deleted for base pairs corresponding to from about base pair 451 to from about base pair 3510 of a wildtype adenovirus genome; and
 - b) a gene expression cassette comprising
 - i) SEQ ID NO: 29;
 - ii) a heterologous promoter operatively linked to i); and
 - iii) a transcription termination sequence.

32. An adenoviral vector in accordance with claim 31 wherein the gene expression cassette is in an E1 parallel orientation.

- 33 An adenoviral vector in accordance with claim 31 wherein the gene expression cassette is in an E1 antiparallel orientation.
- 34. An adenoviral vector in accordance with claim 31 wherein the promoter is a cytomegalovirus promoter devoid of intronic sequences.

5

- 35. An adenoviral vector in accordance with claim 31 wherein the transcription termination sequence is a bovine growth hormone polyadenylation and transcription termination sequence.
- 36. An adenoviral vector in accordance with claim 31 which is at least partially deleted in E3.
 - 37. A cell comprising the adenoviral vector of claim 30.
 - 38. Recombinant, replication-defective adenovirus particles harvested and purified subsequent to transfection of the adenoviral vector of claim 30 into a cell line which expresses adenovirus E1 protein at complementing levels.
 - 39. An HIV vaccine composition comprising purified adenovirus particles of claim 38.
 - 40. An HIV vaccine composition of claim 39 which comprises a physiologically acceptable carrier.
- 41. A method of producing recombinant, replication defective adenovirus particles containing the adenoviral genome of the adenoviral vector of claim 30 which comprises introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and harvesting the resultant recombinant, replication-defective adenovirus.

42. A method according to claim 41 wherein the cell is a PER.C6® cell.

43. A method of generating a cellular-mediated immune response against HIV in an individual comprising administering to the individual a vaccine of claim 21.

5

- 44. A method according to claim 43 which further comprises administration to the individual a DNA plasmid vaccine, optionally administered with a biologically effective adjuvant, protein or other agent capable of increasing the immune response.
- 45. A method according to claim 44 wherein the DNA plasmid vaccine is administered to the individual prior to administration of an adenovirus vaccine.
 - 46. A method according to claim 43 wherein the adenovirus vaccine is preceded by an adenovirus vaccine of a different serotype.
 - 47. A method according to claim 43 which comprises administering and readministering the adenovirus vaccine vector to the individual.
 - 48. An adenoviral vector in accordance with claim 1 wherein the HIV protein is HIV pol or an immunologically relevant modification thereof.
- 49. An adenoviral vector in accordance with claim 9 wherein the gene
 20 expression cassette comprises an open reading frame encoding an HIV pol protein or immunologically relevant modification thereof.
 - 50. A recombinant adenoviral vaccine vector at least partially deleted in E1 and devoid of E1 activity, comprising:

a) an adenovirus cis-acting packaging region corresponding to from about base pair 1 to about base pair 450 and from about 3511 to about 5798 of a wildtype adenovirus genome, and deleted for base pairs corresponding to from about base pair 451 to from about base pair 3510 of a wildtype adenovirus genome; and

5

10

- b) a gene expression cassette comprising
 - a nucleotide sequence selected the group consisting of SEQ ID NO: 1, SEQ ID NO: 5 and SEQ ID NO: 7;
 - ii) a heterologous promoter operatively linked to i); and
- iii) a transcription termination sequence.
- 51. An adenoviral vector in accordance with claim 50 wherein the gene expression cassette is in an E1 parallel orientation.
- 52. An adenoviral vector in accordance with claim 50 wherein the gene expression cassette is in an E1 antiparallel orientation.
- 53. An adenoviral vector in accordance with claim 50 wherein the promoter is a cytomegalovirus promoter devoid of intronic sequences.
- 54. An adenoviral vector in accordance with claim 50 wherein the transcription termination sequence is a bovine growth hormone polyadenylation and transcription termination sequence.
- 20 55. An adenoviral vector in accordance with claim 50 which is at least partially deleted in E3.
 - 56. A cell comprising the adenoviral vector of claim 49.

57. Recombinant, replication-defective adenovirus particles harvested and purified subsequent to transfection of the adenoviral vector of claim 49 into a cell line which expresses adenovirus E1 protein at complementing levels.

- 58. An HIV vaccine composition comprising purified adenovirus particles of claim 57.
 - 59. An HIV vaccine composition of claim 58 which comprises a physiologically acceptable carrier.
 - 60. A method of producing recombinant, replication defective adenovirus particles containing the adenoviral genome of the adenoviral vector of claim 49 which comprises introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and harvesting the resultant recombinant, replication-defective adenovirus.

10

- 61. A method according to claim 60 wherein the cell is a PER.C6® cell.
- 62. A method of generating a cellular-mediated immune response against HIV in an individual comprising administering to the individual a vaccine of claim 59.
 - 63. A method according to claim 62 which further comprises administration to the individual a DNA plasmid vaccine, optionally administered with a biologically effective adjuvant, protein or other agent capable of increasing the immune response.
 - 64. A method according to claim 63 wherein the DNA plasmid vaccine is administered to the individual prior to administration of an adenovirus vaccine.

65. A method according to claim 62 wherein the adenovirus vaccine is preceded by an adenovirus vaccine of a different serotype.

- 66. A method according to claim 62 which comprises administering and readministering the adenovirus vaccine vector to the individual.
- 67. An adenoviral vector in accordance with claim 1 wherein the HIV protein is HIV nef or an immunologically relevant modification thereof.

5

10

15

- 68. An adenoviral vector in accordance with claim 9 wherein the gene expression cassette comprises an open reading frame encoding an HIV nef protein or immunologically relevant modification thereof.
- 69. A recombinant adenoviral vaccine vector at least partially deleted in E1 and devoid of E1 activity, comprising:
 - a) an adenovirus cis-acting packaging region corresponding to from about base pair 1 to about base pair 450 and from about 3511 to about 5798 of a wildtype adenovirus genome, and deleted for base pairs corresponding to from about base pair 451 to from about base pair 3510 of a wildtype adenovirus genome; and
 - b) a gene expression cassette comprising
 - a nucleotide sequence selected the group consisting of SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13 and SEQ ID NO: 15;
 - ii) a heterologous promoter operatively linked to i); and
 - iii) a transcription termination sequence.
 - 70. An adenoviral vector in accordance with claim 69 wherein the gene expression cassette is in an E1 parallel orientation.

71. An adenoviral vector in accordance with claim 69 wherein the gene expression cassette is in an E1 antiparallel orientation.

72. An adenoviral vector in accordance with claim 69 wherein the promoter is a cytomegalovirus promoter devoid of intronic sequences.

5

10

- 73. An adenoviral vector in accordance with claim 69 wherein the transcription termination sequence is a bovine growth hormone polyadenylation and transcription termination sequence.
- 74. An adenoviral vector in accordance with claim 69 which is at least partially deleted in E3.
 - 75. A cell comprising the adenoviral vector of claim 68.
- 76. Recombinant, replication-defective adenovirus particles harvested and purified subsequent to transfection of the adenoviral vector of claim 68 into a cell line which expresses adenovirus E1 protein at complementing levels.
- 77. An HIV vaccine composition comprising purified adenovirusparticles of claim 76.
 - 78. An HIV vaccine composition of claim 77 which comprises a physiologically acceptable carrier.
 - 79. A method of producing recombinant, replication defective adenovirus particles containing the adenoviral genome of the adenoviral vector of claim 68 which comprises introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and harvesting the resultant recombinant, replication-defective adenovirus.
 - 80. A method according to claim 79 wherein the cell is a PER.C6® cell.

81. A method of generating a cellular-mediated immune response against HIV in an individual comprising administering to the individual a vaccine of claim 78.

- 82. A method according to claim 81 which further comprises

 administration to the individual a DNA plasmid vaccine, optionally administered with
 a biologically effective adjuvant, protein or other agent capable of increasing the
 immune response.
 - 83. A method according to claim 82 wherein the DNA plasmid vaccine is administered to the individual prior to administration of an adenovirus vaccine.

10

15

- 84. A method according to claim 81 wherein the adenovirus vaccine is preceded by an adenovirus vaccine of a different serotype.
- 85. A method according to claim 81 which comprises administering and readministering the adenovirus vaccine vector to the individual.
- 86. A multivalent adenovirus vaccine composition comprising recombinant, replication-defective adenovirus particles, wherein the adenovirus particles are harvested and purified from a cell line expressing adenovirus E1 protein, and wherein the particles are harvested subsequent to transfection of the cells with an adenoviral vector or vectors in accordance with claim 9; said vector(s) comprising a gene expression cassette or cassettes comprising nucleotide sequences encoding HIV proteins selected from the group consisting of:
 - a) gag, pol, and nef, expressed independently from three individual vectors;

 b) gag, pol, and nef, expressed independently from one vector with the encoding nucleic acid sequences operatively linked to distinct promoters and transcription termination sequences;

- gag, pol, and nef, expressed via two vectors, one expressing a polnef fusion, and another expressing gag;
- d) gag, pol, and nef, expressed via two vectors, one expressing a gagpol fusion and another expressing nef;
- e) gag, pol and nef, expressed via two vectors, one expressing a nefgag fusion and another expressing pol;
- f) gag, pol, and nef, expressed via one vector expressing a gag-polnef fusion;
- g) gag and pol, expressed independently from two individual vectors;
- h) gag and pol, expressed independently from one vector with the encoding nucleic acid sequences operatively linked to distinct promoters and transcription termination sequences;
- i) pol and nef, expressed independently from two individual vectors;
- j) pol and nef, expressed independently from one vector with the encoding nucleic acid sequences operatively linked to distinct promoters and transcription termination sequences;
- k) nef and gag, expressed independently from two individual vectors;
- nef and gag, expressed independently from one vector with the encoding nucleic acid sequences operatively linked to distinct promoters and transcription termination sequences;
- m) gag and pol, expressed via one vector expressing a gag-pol fusion;

5

15

n) pol and nef, expressed via one vector expressing a pol-nef fusion; and

- o) nef and gag, expressed via one vector expressing a nef-gag fusion.
- 87. A multivalent adenovirus vaccine composition in accordance with claim 86 wherein the gag-pol fusion consists of SEQ ID NO: 39.
 - 88. A multivalent adenovirus vaccine composition in accordance with claim 86 wherein the fused sequences have the encoding nucleic acid sequences operatively linked to distinct promoters and transcription termination sequences.
- 89. A multivalent adenovirus vaccine composition in accordance with

 10 claim 86 wherein the fused sequences have the encoding nucleic acid sequences

 operatively linked to a single promoter; and the encoding nucleic acid sequences

 operatively linked by an internal ribosome entry sequence ("IRES").

Original Adenovector Construct:

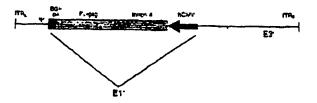


Figure 1: Original HIV-1 gag adenovector.

Sequence of the open reading frame for FL-qaq (human codon optimized)

atgggtgctagggcttctgtgctgtctggtggtgagctggacaagtgggagaagatcaggctgaggcctggtgg caagaagaagtacaagctaaagcacattglgtgggcctccagggagctggagaggtttgctgtgaaccctggc agctgaggtccctgtacaacacagtggctaccctgtactgtgtgcaccagaagattgatgtgaaggacaccaag gaggecciggagaagattgaggaggagcagaacaagtccaagaagaaggcccagcaggctgctgctgctgc acaggcaactccagccaggtgtcccagaactaccccattgtgcagaacctccagggccagatggtgcaccag gecatetecceggaccetgaatgeetgggtgaaggtggaggaggaggaggaggaggetteteccetgaggtgatecc catgitctctgccctgtctgagggtgccacccccaggacctgaacaccatgctgaacacagiggggggccatc aggetgecatgeagatgetgaaggagaceatcaatgaggaggetgetgagtgggacaggetgeateetgtge acgctggccccattgccccggccagatgagggagcccagggggctctgacattgctggcaccacctccaccct ccaggagcagattggctggatgaccaaccaccccccatccctgtgggggaaatctacaagaggtggatcat cccticagggactatgtggacaggttctacaagaccctgagggctgagcaggcctcccaggaggtgaagaact ggatgacagagaccctgctggtgcagaatgccaaccctgactgcaagaccatcctgaaggccctgggccctg gctgaggccatgtcccaggtgaccaactccgccaccatcatgatgcagagggcaacttcaggaaccagag gaagacagtgaagtgcttcaactgtggcaaggtgggccacattgccaagaactgtagggcccccaggaaga agggctgctgggaagtgtggcaaggagggccaccagatgaaggactgcaatgagaggcaaggccaacttcctg ageiglaccccctggcctccctgaggtccctgtttggcaacgacccctcctcccagtaaaataaagcccgggca gat (SEQ ID NO: 29)

Figure 2

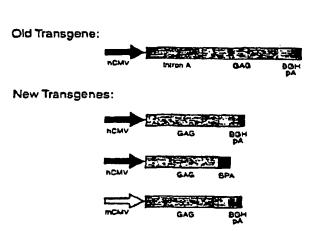


Figure 3: Diagrammatic representation of the original HIV-1 gag transgene and the series of new transgene constructions.

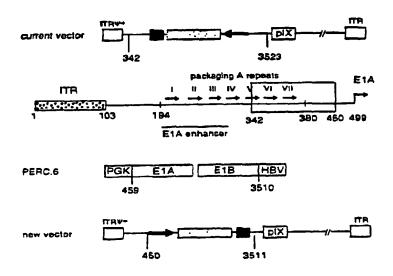


Figure 4: Modifications made to the current adenovector backbone in the generation of the new vector.

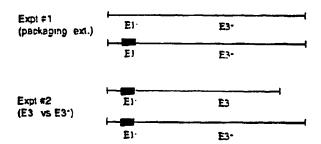


Figure 5: Virus mixing experiments to determine the effects of the addition made to the packaging signal region (Expt #1) and analysis of the effects of the E3 gene on viral growth (Expt. #2). The red bars denote the region of modifications made to the E1 deletion.



Figure 6: Autoradiograph of viral DNA analysis following viral mixing experiments (expts. #1 and #2) as detailed in the text.

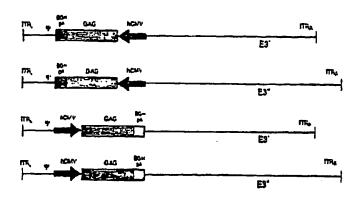


Figure 7A: hCMV-FLgag-bGHpA adenovectors constructed within the "MRK" backbone. E1 parallel and E1 antiparallel transgene orientation within the E3- and E3+ backbones were constructed.

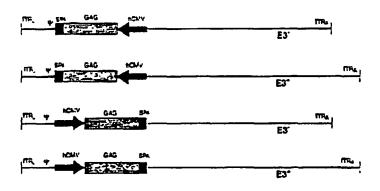


Figure 7B: hCMV-FLgag-SPA adenovectors constructed within the "MRK" backbone. E1 parallel and E1 antiparallel transgene orientation within the E3- and E3+ backbones were constructed.

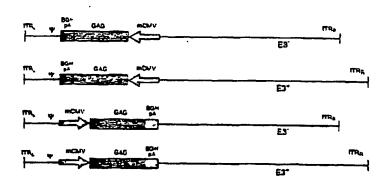


Figure 7C: mCMV-FLgag-bGHpA adenovectors constructed within the *MRK* backbone. E1 parallel and E1 antiparallel transgene orientation within the E3- and E3+ backbones were constructed.

Plasmid mixing expt: (orientation)

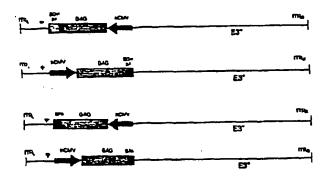


Figure 8A: Effect of transgene orientation

Plasmid Mixing expt: (poly A signal)

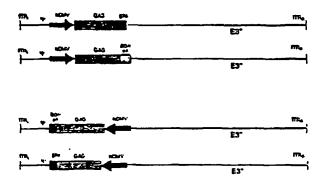


Figure 8B: Effect of polyadenylation signal



Figure 9: Viral DNA from the four Adgag candidates at P5, following BsfE11 digestion.

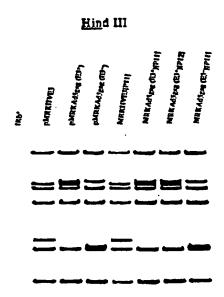


Figure 10: Viral DNA analysis of passage 11 and/or 12 of MRKHVE3, MRKAd5gag and MRKAd5gag(E3-).

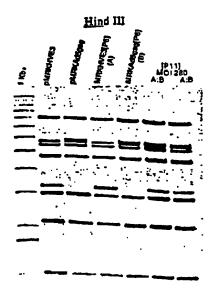


Figure 11: Viral DNA analysis (*Hin*dIII digestion) of passage 6 MRKHVE3 and MRKAd5gag used to initiate the viral competition study. Last two lanes are passage 11 analysis of duplicate passages of the competition study (each virus at MOI 280 vp).

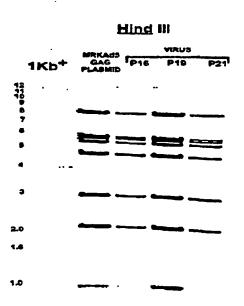
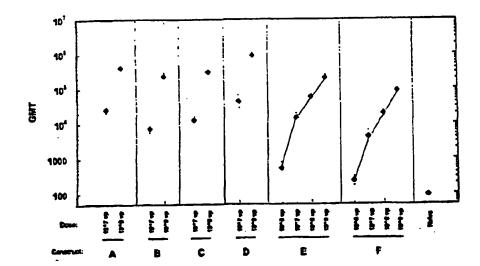


Figure 12: Viral DNA analysis by *Hind*III digestion on high passage numbers for MRKAd5gag in serum containing media with collections made at specified times. The first lane shows the 1 Kb DNA size marker. The other lanes represent pre-plasmid control (digested with Pac1 and *Hind*III), and MRKAd5gag virus continually passaged to P16, P19 and P21(serum containing media).

Figure . Serum anti-p24 Levels at 3 Wks post i.m. immunization of balb'c mice (n=10) with Varying Doses of Several Adgag constructs: (A) MRKAd5gag (through passage 5): (B) MRKAd5 E3 hCMV-FLgag-bGHpA; (C) MRKAd5 E3 hCMV-FLgag-SPA; (D) MRKAd5 E3 mCMV-FLgag-bGHpA; (D) research Lot (293 cell-derived) of Ad5HIV-lgag; and (F) clinical lot (Ad5gagFN0001) of Ad5HIV-lgag. Reponde are the geometric mean titers (GMT) for each cohort.



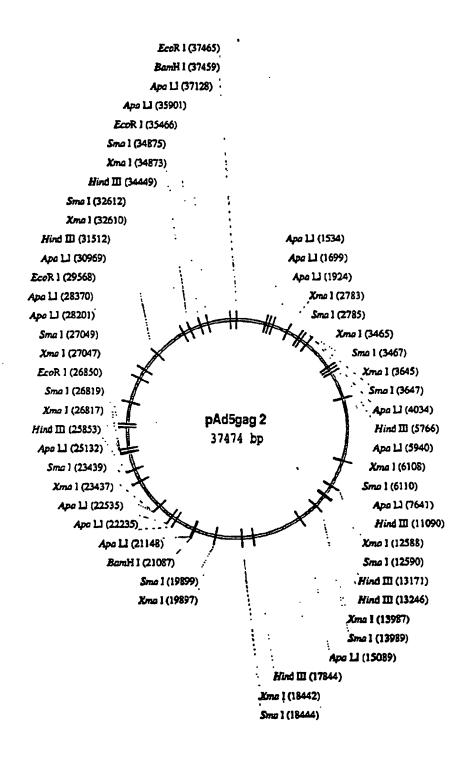


Figure 14

AGGGCTTCTG TGCTGTCTGG CCATICKGCAC כשמענינייד TENCTOCATA ATAGTATACT: CCTANOXCOTI: CACACCCCCC COCCANNUT CANCINCTICITY TO CCATATCATY CCTATACTAT CATTAGETTCA GTANTCAAGT AATCACCTAT **NGTACATOTA TCATTATAGAT** TATCATATO COCHOLOGO AGAGGTTOKO Terceveer CAGGTATCTT ACHGCAGTTA GCATCAACCG DATTTCCAAG CTANAGETTC CCAAATGGGC COTITACCCO CTCCATAGAA CCANATGCAC ATCCAACATA ACATCAAGTO TOTACTICAC CCTACTTOGG **OCCARTITICO** COSTANAGE CONTINCOTO TACOTTOTAT ATTACGGGGT **FAATGCCCCA** TCACCTCAAT TCACOFFEE ACTOCANANA COCUMOCOCC בכנינונינינינינים TRACATETAE CATOGOTOCT GGACTTTGAC CCTCANACTO TACCTAACCT ATTACTACTOR CCCCCCCAT GROCCOOTA ATCCCACTIT TACCCTGANA CCCCATTGAC GOODTANCTO CICITATICAC GACAAAACTO GTANGATTO CATTCTARAC ATCCATTGCA TATCATTAGE ACTICOCAGE TOMOGRACA GACTICACOOO CTCAGTGCCC CTCCCANNO CACCOTITIC THENCACHE AACACTESCA TANACTIOCCC CATANATIZE ATTIGACIOO ATAGACKETT AACAACTCCO THUTTICACIC COGTARGTGC CATTITADACE COCATIONET TATTATTATA GROCECOCO 2020022022 CTACTTATTA TACAACTGTA ACTAATAACT GATCAATAAT CTRRICTERACE DECENACIONE CONCINCEN ACATGACCTT TICTACTICION TATCCCCAAA GCCATCCACG TATAAACAGA TECEDGEGEE CONTRACTOR CACCACCTC ACCENTINAT TIGISTACAT TCIXCTUCCTA CHATTANCCCA ANATISTICGT TTTACAGCA COCAGITY OF COGNICITY CHRICTABILAG מאככתיאכיתים CTATTTACKE ATACCCAPTCA ATICICALITATION GPCATGITAGE TACCCCXCACC GCCTGGAGAGAC TATGCCCAGT ATGATAATKIA ACTIVITATION TRETATTACT AACACATGTA GTANATTIGG ATATHERET ATAATAATAT TCATTATTCA CCCTGAAAGG CASCACTITICS GTTTAGTGAA CCGTCAGATC TANCCCACCT CCCCACCCTA CENTRITICITA CCTACAACAT ATATATATA CHICKICCHIT CAMINITICANA ATCTTCACAT ANTYRICTICAL TACCOGGG ANTCACATAGA CCCCTTGCCAT CASTACATCA ACTUTATION TCACACCCCC TATY, W. W. TATAT CCANANTCCT **OFMCTGCAG** COCCANANCO CANATICACTT PUTCHTACTC GCCCAGTTT CTAATGRACIC ACTEACOGEA TCAATGCCAT CTANATICCC CATTTACCOS CCCCTTTTCC CAMATCANC GETTERGITE ACACANTIGAG CCCIGGTCAM CATTACCGCC CATTGACGTC TATTITICAGAT ATANANCITA TGATGATAGA ACTACAACGT COTTITAGG COCMATGTAT **GOTACCACTA** GTTTTGGCAC ACCAGAGCTC STOCCACCCT CCAGATATAT TCCTCTCGAG **GCUTTACATA** TAACGCCAAT AGGGACTITIC TCCCTCAMO GTCAATCACG CAGTTACTOC CCATCCTCAT CAMACCOTO ACTTATTAM ANACTRICECAR TCATCTCCAA AGTACAGGTT TAATATACT ATTATATA ההכסואתיוני CCCCC TTCAC ATT TRIVERS TAMARCON TCANTANTT THYCOTOTH TACCTCAAGG CCCTATTOAC ATCCCTATTA AGAGTCCACA TTATATTOOC AATATAACCO ATCOACTICC ATTOCOUTA GOCATAACTO TACCCATANT ATCCCACTT TACCCTCAMA GOTCTATATA TICACTITAG TICHTANTTA ACATCATCAA CATCATCACA GOAAGTGACA CCTTCACTOT ANGTONNATC **TCTCAGGTGT** ANGANTEMAT TOTACTAGET GENGTAGNET COTATTACT CCCCCACTG CCACATGROT CHTATHETEC CAGGIGITH GTCCACAAAA ATAITGENCAL TACCCATAT ATCCCCCTATA GITCCCATAG CAMOGOTATO CANGITACOCC GITCATGCGG CCATAATCAG NTTCACCOTCA PACTCCAGE **FACOOTOOGA** COTOTACACA CANTARCAGO **TATACATOTA** COCCOCOTORC 1101 601 701 108 901 1001 201 501 101 101 201 5

CATHOLOGICALIANGO CACGACTURA ACCAGAM'AA CCAGATOCTC ACCACAGACC CONCCINITION renemon GENCTACCAC CACCTCACCT TCCCCAAGAC COMOGRECET AGGCTCTGAG TCCCAGACTC ACCTCCAGO CCTCCAGGGA ATTOROGOAGO TAACTCCTCC TOGAGGICCC GCTAAAGCAC ATTGTGTGGG THINKITHE MEACAGAIN CTICATERE TANCACCTET CCCTOCAMC GANCTACCCC ATTOTOCAGA CACCITICACA CACACOTITIO CCANATAGGE CCTOGAGANG CONTECUES GENERALE **GTACCCACGA** TANCACACCC ACTUTAGATO CCATTACGTG CTCCAGCCT CACCATTACTIC Trittonhitt GRACECTATIC CACTICCTOT ACKET TECCE A MINNETACAA הכדנסמצכלת CITTENNYAGALA GCCTAACAGG CCACTCCOCA CCACCGTTCT CCFFCCCFFCFA AT: WE THO AACTICACET CATTOGNACIO COGATTCCCC COLUMN TO THE PARTY OF THE PART CCACACACAT CHETYKACCA GAMPATTEAT TOXCACACIC EMOSTICITIC TICCONSTCO TCCGACGACG ACCONTATICGS TCTCAGGGGT AGACTCCCCA CACACCTRACT **OCTRINOGENET** CTANCCTTCC GOGAACOOTO AGAAGATCAG OCTOGAGACC CGACCTCTOG ACCENTANCE OTOTCACCOA TOGGACATGA ARXITYSCITAC CCCTTGCCAC TCTTCTAGTC MODECCAGE CHECGEOGECE CHOPPICACCC ACCETODOCE TOCCACCOGA CAGCCGCCCC CACAAGTGGG CACAGTOGCT MACCACACT DOGACATOTT STECHERNS THOCHORA CCCTOTACAA CCGATCCAGC DOCTROCTEG POSTGAGGTG ACCACTCGAC 1601 1301 1501 1401

Figure ISA

PMRKAITGAG HER682

1101	CACCAGGCCA	•	- '	CCCTICACTICA	ACT TO THE TANK	FRAGABARACE	THETECOGIG	AGGTGATCCC	CATOTTCTCT	GCCCTGTCTN
1801	ACCORD	CCCCCARGAC	CHEMACAPTA	Transfer ACT	NCHARACON CT	CAME ACTOR OF	MICHIGACIACIAC	TCCACTAGOO	GTACAAGAGA	CCANTINCAGAL
•	TCCCACGGTG	_	_	ACCIAL TITITO	TUNTIVOK	CTACTACCAC	COTACCTCTA	CCACTTCCTC	TOGREGITAC	Tremerate :
1901	TCACTCGGAC	ACCETCANC	CTRIMENIES	Transcinctage	מנענגנוניאנינ	אנאיאיאראיא	CACCACCACA	TCTGACATTIC	CTOSTACTAC	
	ACTICACCOTO	TCCOACOTAG	GACACCETCA	ACCURACTA	ورعوبته بالمراج	TITACTURE	CYNCTOCICO	AGACTOTAAC	GACCOTGGTG	OMACHICANO
2001	CAGGAGCAGA	Traccraaat	GACKIANTANC	CCCCCATC	ATTENDED	ANTITACAAG	AFFTERSATEA	TECTAMORECT	GAACAAGATT	GIVACASATURE
	Greencores	ANCCOACCTA	CTCGTTCTTC	CHRECETAGE	מאנאנוכנענד	TTAGATGTTC	TCCACCTAGE	AGGACCCGGA	CTIGHTCTA	CACTCCTACA
2101	ACTCCCCCAC	CHECATECTIO	GACATCOVANC	ACCIONATION	GWACKTHE	ARCGALTATG	TGGACAGATT	CTACAAGACC	CTCAGGGCTG	ACCAPOCCT
	TOAGGGGGTG	GAOGTAGGAC	CTCTACTCCC	TCCCCCCCCTT	CCTCCGCCAAAG	TCCCTGATAC	ACCTIGACCAA	GATGTTCTGG	GACTCCCGAC	TCUTCOGA
2201	CCAGGAGGTG		TCACAGACAC	CCTOCTCRETG	CACAATGCCA	ACCCTGACTG	CAMBACCATC	CTGAAGGCCC	TOGRECETOR	TOCCACICTO
	OGTECTECAC	TICTIONCCT	ACTROPOLICIO	OGACTACCAC	CTCTTACCCT	TOGGACTOAC	GENCHOOTAG	GACTICCOOG	ACCCCCCCCACC	ACCOUNCY.
2301	CACCACATCA	•	CCACCACATIC	GRAGGATCING	CTT.ACAARGC	CACCOMMETE	GCTGAGARCEA	TOTOCCAGGT	GACCAACTEC	GCCACCATC.
	CICCICIACI	ACTIOTCOGAC	GOTCCCCCAC	CCCCCOGGAC	CACTUTACES	GTCCCACCAC	CGACTCCOGT	ACAGGGTCCA	CTGGTTGAGG	COCHOCTACI
2401	TCATOCAGAO	GOGCANCTIC	ACCIMACCAGA	GEANGACAGE	CAACTECTTC	AACTIGHTCCA	AGGTGGGCCA	CATTGCCAAG	NACTOTAGGG	כבכבבישאי.
	ACTACOTOTIC	CCCGTTGAAG	TCCTIGGICT	CCTTCTGTCA	CTTCACGAAG	TRIACACCUST	TCCACCCAGT	GTAACGGTTC	TTGACATCCC	OCCUPATION IN
2501	GARGOCTOC	TOGAMOTOTO	GCANGGAGG	CCACCAGATO	AACHACTECA	ATCAGAGGA	COCCAACTIC	CTCCCCAAA	PCTGGCCCTC	CCACABCANA
	CTTCCCCACC	ACCTTICACAC	CONTROCACCE	GGTGGTCTAC	Trecreacet	TACTCCCT	CCRGTTCAAG	GACCOSTITI	AGACCOGGAG	GCTCTTCCC
2601	AGGCCTGGCA	ACTICCTCCA	GTCCACGCCT	GNGCCCACAG	CCCCTCCCCA	GGAGTECTING	AGGTTTGGG	AGGAGAAGAC	CACCCCAGC	CAUSANGCAR
	TOCOGRECOS	TCAACCACCT	CAGGTCCGGA	CTCCCCCTCTC	GROCAGINGT	CCTCAGGANG	TCCAMCCCC	recrement	GTGGGGGTCG	GICHOGIC:
										Byti
2701	AGCCCATTGA		TACCCCCTRIG	CENCICTOAR	GRECEINFILE	OPERATOR	CCTCCTCCCA	GTANNITAAA	ОСССОВЯЕЛЬ	ATCTOCTOR:
	TCOOGTAACT	OTTOCHOOAC	ATOCOCCACC	(XYACCOCTC	CACACACAAA	CCTITICATION	CCACCACCCT	CATITITATITE	COCCCCTC	TACIACCACA
2801	CCTTCTAGT	OCCADICATIC	TOTICITIO C	CCCTCCCCCG	TOCCITICON	DARCETOGAA	COTOCCACTO	CCACTOTCCT	TTCCTAATAA	ANTCACOUNT
٠	DOAMGATCAA	COCICOCIAG	ACANCANACG	COCAGGGGGGGC	ACCICANGGAN	CTGGGACCTT	CCACGGTGAG	GCTCACAGGA	AAGGATTATT	TTACTCCTTT
,										Sith
2901	TTGCATCGCA	TTOTCTOAGE	AGGTGTCATT	CTATTCTORG	CKROTROPOSTIC	RECORDACA	CCAACCTATA	CCATTICCGAA	GACANTAGEA	GGC:ATTCCTFGG
	AACGTAGCGF	AACAGACTCA	TCCACAGTAA	GATAAGACCC	CCCACCCCAC	CCCGTCCTGT	CGTTCCCCCT	CCTANCCCTT	CIGITATOGE	CCCTACGACC
			P							
	•		Asd							
3001	OTATOCOGTO		CCGATCGCG	COCCUTACTO	AAATGTGTG	CATCHART THA	AGGGTGGGAA	AGAATATATA	AGGTGGGGGT	CITATICIAL
	CCTACGCCAC	CCGAGATACC	OOCTARICERS	OCCIOCATICAC	TITACACACC	CCCACCGAAT	TCCCACCCTT	TCTTATATAT	TCCACCCCCA	GANTACATCA
	٠								Sphi	
3101	TITOTATICIO	TTTTGCAGCA	פכבמכבעכנפ	CCATRIMICAC	CAACTOCTET	GATGGAAACA	THOMPHOLIC	ATATTTEACA	ACCIOCIATOR	CCCCAINSSIC
	ARACATAGAC	MAACCITCUT	בססטטטנעטנ	GGTALTER	CTTCACTCAAA	CTACCTTUGE	MICHETEGAG .	TATAAACTOT	TOCOCOTACO	COCCERCENT
3201	COCOCTCCCT	CAGAATCITCA	איזידי איציד	CATHGATTA	وديدرينيد	THECTHINA	כובבאינדאנים	THANCETACG	AGACCGTGTC	TOCARCOCCO
	GCCCCACGCA	STICHTACACT	ACCURAGGIC	CTAACTACCA	CCCATATACACAC	ACCORDERATION	CACATGATTS	AACTOGATOC	TCTOCCACAG	ACCTTGCGGC

tique 150

PMRKAdSgag MER682

	6	5		č						
1101	THOUSAGE C	CAGTOROGE	CALCACTE	مستسمد	כראינייקייני	האבאדונהוני	Activitation	CHITTCHISAG	CCCXCTTGCA	AACAK:TCCAG
	MCCTCTGAC			ביד:ריא:יי:דנ:	.KKKLKEMIK)	מינבוואליאנאנ	TEACTENAAC	GANARACTC	CCACCOAACOT	THEFCACGIC
3401	CHICCOOPIC	ATCCGCCCCC	CATCACARGE	TCACKER TCT	TITYREACAN	THEMATACETE	TEACTECTARA	ACTTANTOTO	GENTENCE	ACK:TV:TTN::A
•	GANGGISCAAG	_	CTACTOTACA	ACTRICCENCA	ANANCOTOTT	MCCTMGM	ACTORNICECT	TGANTTACAG	CAAAGAGTCO	TCCACAAN T
3501	TCTGCGCCAG	CACCOTTICTO	CCCTTAACAC	TRUTTUCCUT	CCCAATIACE	TTYWWLAT	ANATANAAAA	CCACACTETO	TTAGATTE	GATCAAGCAA
	AGACGCGGGTC	GTCCAMGAC	GOOD ACTIONS	AAGGAAGGAAAA	GRATTACKE	AMTITRITA	TITATITIT	OCTETOAGAC	AAACCTAAAC	CTAGITICAL I
3601	GIOTCHACT	GICHTAFT	ACCOCHETE	دىتىتىدىدىنىد	ARRICCCYNEA	CCASCASCACT	CONTCOTTGA	CONTRICTOR	TATTITICC	ACCACCTANT
	CACAGAACGA	CACANATANA	TCCCCAAAAC	GCCCCCCCCA	TCCGGGCCCT	CKTCTCCAGA	GCCAGCAACT	CCCAGGACAC	ATABABABGG	TECTGCACE
							Pstl			
3701	ANAGGTGACT	CTOCATOTIC	AGATACATGG	CCATANCEC	CHETETRACE	THEMOGIANSC		ACCTICATEC	TOCOCOCTOO	ACTICTAGAT
	TITICCACTICA	GACCTACANG	TCTATGTACC	CGTATTCGTAG	CACIANTACION	ACCTCCATCG	TOCHGACGIC	TCGAAGTACG	ACOCCCCACC	ACAACATICTA
3801	GATCCAGTCG	1 TAGCAGGAGC	CETOSACCAO	GTGCCTAMA	ARCTUTACA	GTACKAAGCT	GATTGCCAGO	GOCAGGCCCT	TCOTCTANOT	GPTFACAAN:
	CTAGGTCAGC	: Ancorcence	CGACUTICAC	CACGGATTTT	TACATAMET	CATESTICEA	CTANCOCTICC	CCGTCCGGGA	ACCACATTCA	CAMATGHTAC
1961	COCHIMOCT	r occarocero	CATACCATORS	CATATUACAT	GCATCTTISCA	CRUTATION	ACKTERCOCTA	TOTTCCCAGC	CATATCCCTC	CORRESTIV'A
	OCCAATICGA	-	GTATGCACCC	CTATACT:TA	CCTATAACCT	CACATAMAA	TECANCEGAT	ACAAGGGTCG	GTATACCICAC	ACCCCTANGE
4001	TOTTOTOCAG	ANCEACEAGE	ACAGICATIC	COGTEXCACTT	GGCMATTIG	TCATISTAGET	TACAACCAAA	TGCGTGGAAG	AACTTGGAGA	CCACCCTTGTVI
	ACACACOTC	•	•	CCCACCTGAA	CCCTTTANAC	ACTACATEGA	ATCTTCCTT	ACCCACCTIC	TTCAACCTCT	GCCCCLAACAC
4101	ACCTCCANGA	1 TTTTCCATOC	ATTCGTCCAT	AATTATTAA	ATTAPACTICAL	TANGONG CONC	CTYCCCCANG	ATATTACTOG	CHICACTANC	CHEATAGHTO
	TOGAGGITCT	P ANAGOTACO	TANGCAGGTA	TTACTACCGT	TACCCOCATAG	تحديدحيرجو	CACCCOCTTC	TATAMCACC	CTACTGATTO	CARTATCAN
4201	TOTTCCAGGA	TOAGATEGIE	ATNAXCATT	TTTACAMAGE	GCTATICCANG	CHATCHCACAC	TCCCCTATAL	TOGTICCATC	COCCCAGGG	GCGTAGTTA
	ACAAGGTCCT	-	-	AAATGTTTCG	COCCUBCENC	CCACGGTCTG	ACCCCATATT	ACCAAGGTAG	OCCOGENICAC	CCCCATCAA'n:
4301	CCTCACAGAT	•	CACCETTAGA	CTTCAGATOG	GENERATION	TETACETORS	COCCCONTICAL	GARARCOGIT	TCCOGOGTAG	GCC:ACIATICIA
:	GGAGTGTCTA	=	_	CAAGTCTACC	CCCCTAGTAC	AGATGGACCC	CCCCCTACT	CHITTICCCAA	AGGCCCCATC	CCCTCTAKTIL:
										<u> </u>
4401	CTCCCAACAA	ACCAGOTTCC	TCACCACCTO		CANCYOGINAS	CCACTTACCS CARCYOGING GCCCGTAAAT CACACCTATT	CACACCTATT	ACCOCCTOCA	ACTOSOTAGIT	AACACACTY:
3	CACCCTACT	-			CHECOCCACC	COCCUNTIA	GTCTGGATAA	TRECCEMENT	TONCCATCAA	PICTCTCGAC
	Pen					-	-			
4501	CADCIDCCOT	CATCCCTGAG	CAGGRAGGCC	ACTICGITIM	ACTTOGITIM GCATGICCCT	CACHCCATATO	THITICACTICA	CCAAATCCOC	CAGAAGGCGC	TCCCCCCCCA
!	GTCCACCOCA	-	Grececes	TCAACCANT	CCTACACIAGA	CTGAGGGTAC	ANANGOGACT	GCTTTTAGGCCG	OFCTFCCGCG	ACCOCCOON
							Hip?			
4601	OCCURTACCAG	# TTCTTOCANG			אנומאפאנונו		CCATTENT		CCANGCAGIT	CCAGGCGGTC
	CUCTATION	: MOMOUTE	CITICOTTICA	ANAGINGEC	AMCTICITATIO	NAR.CARCATC	CGTACGAAAA		GGTTCGTCAA	משכנפכניי
4701	CCACAGCTCG	S STEACCTOCT	CTACGGCATC	TOTATOCARC	ATATCTCCTC	GENERALING	PTGTAGCGGC	THEOTOTA	CACCAGTAGT	כשיומכוכהיי
	GOTOTCOAGC	: CAGTOCACCA	CATCCCCTAG	ACCTAGGETCC	TATACACTING	האאינהרוכ	AACCCCCCCC	MACCACAT	GCCGTCATCA	CCC MCGMIN'N
4801	CCAGACGGGC	: CACCOTCATO	PCT TTCCACG	CARTA NAKA	CCRUTATIONS	הדאנהתנואס	TCACCCTCAA		ccasscrace	COLTICATION.
	GENCTIFICACE	S GREECAGTAC	AGANAGGTOC	הכתבכבוכבכנת	CONTRACTIC	CATCAGACCC	AGTICCACTIT	CCCCACGCCA	COCCCOVEDE	CCCACCIGGT"

figure 15c

PMRKAdSgag MER682.

4901	amacactro	AGCTORICC	TACTOTACE	GAAGACA TER	CYCLETROIS	CETTATE	CHILLIANS	CATPURER	A CALLEST A CALL	
	CCACGCGAAC	TCCGACCAGG	_				כנישיוניניאונ	GTANGTOOT	ACCACACTAT	
2001	TCCGCGCGT	GOCCCTIGOC	OCCICACITY	CONTRACACA	אינויאיניאיניאר		TYTCACACTET	TYTHOOCCOTA	GAGCTRASS	
	AGGCCCCCCC	CCGGGAACCG	CCCONCGANC	COCANCUTCO	Tecaraca	(# Tricked	ACCITCITIONA	ACTCCCCCAT	CTCGAACCCG	
5101	CCGATTCCGG	GONOTAGGCA	TCCCCCCCC	AGGICCICIACA	GACGERTAN			CICTOCCCOT	TOTABLETICAN	
	GCCTAAGGCC	CCTCATCCGT	ACCCCCCCCC	Ţ.	CTRCCAGAGC			GAGACCOCCA	AGCCCCAGTT	
,									flat	
5201	Teecechiae	-		TCTCATTICC	ATVINAZONOT	CITCCACGCITC	GCTGACGAAA	AGGCTSTCCG	TOTCCCCOTA	TOTCCCCTA TACACATY;
	AGGGGGTACG	AANAACTACO	CANGANTED	NGACCAMAGG	TACTITIZATEA	CARCTACGAG	CCACTGCTTT	TCCGACAGGC	ACAGGGGCAT	ATCTCTCAM:
		Karot								•
5301	ABAGGCCTGT	CCTCCACCCC	TOTACCIACOG	TECTOCACOT	ATAGMACTC	GRACCACTOT	CACACAAAGC	CYCOCONCCA	OCCAGGACO	AACROMOGETA
	TCHCCGGACA	DOMOCTOCCC	ACANGGEREC	ARGARGACCA	TATCTTICAG	CC.TROTGAGA	CICIOTITCC	OAGCCCAGGT	CCGOTCCTCC	
5401	ACTOGGAGGG	OTACCOCICO	-	GOOGSTECAC	TURCHUCAGO	MCTCAAGAC	ACATGTCCCC	CICITOGGCA	TCMGGMGO	TOATTOSTED
	TCACCCTCCC	CATCOCCAGE	AACACOTCAT	CCCCCAGGTG	MACHINACTEC	CACACTTETO	TOTACARCOG	GAGAAGCCGF	AGTICCTICC	ACTACCAM
5501	CFACCIOTAC	OCCACOTOAC	-	TGANGGGGGGG	CTATAAAARG	GOCTGGGGGC	COUNTOINCE	TCACTCTCT	CCCCATCCCT	GICTOCOACO
	CANCCACATIC	COGTOCACTO	GCCCACAAGG	ACTICCCCCC	CATATTTEC	CCCACCCCCG	CCCAARCAGG	ACTGAGAGAA	DOCOFABCOA	CAGACCCTCC
5601	OCCADONOL	GOOGTGAGTA	CICCOTOTOA	AAAGCGGGCA	TENCTICE	GCTAAGATTG	TCAGTTTCCA	AAAACGAGGA	COATTICATA	TICACCION.
	COGITCGACAA	CCCCACTCAT	GAGGCAGACT	TITCGCCCGT	ACTGAAGACG	COATTCTMC	ACTCAAAGGT	THIRTICCT	CCTAAACTAT	ANCTICACCT
							Porcell			
5701	CCCCCCCTCAT	OCCUPIONO	ETGCCCCA1	CCARCTONIC	AGAMAGACA	ATCTITUTE	TGTCAAGCTT	GENGERAAR	GACCESCARACIA	Ca a a representative
1	DOCCCACTA	COCHANCTICC							CTOCCATCT	CCCCAACCT
	•			•	Percel					
5801	CACCAACTTO	OCCUNTOCAGE	GCACKOTTTG	GETTERORGO	CCATCACCC	ACTICITIONS	CGCGATGTT	AGCTGCACGF	AFTEGEORGE	AACCCACCCA.
	OTCOTTOAAC	COCTACCTOS	COTCCCAAAC	CANARACIOC	מכדאמככמכם	CCACATAACCC	GCCCTACAAA	TECHEOTOCA	TAAGCGCGCG	THECOTECOJ
5901	CATTCOOGNA	AGACCOSTGGT	GCOCTCOTCO	GOCACCAAST	CCACRCRCCA	ACCECCION	TCCAGGGTGA	CANCOTCAAC	OCTOOTGOCT	Accretector
	GIMAGCCCTT	TCTGCCACCA	COCCAGCAGC	CCGTGGTCCA	CGTCCCCCCT	TCACCCCANC	ACCITECTACT	GITCCAGTIG	CCACCACCCA	TOCACAGOL
6001	GTACOCOCTC	GITGOICCAG	CAGAGGCGGC	COCCCTINACO	CGARCAGAAT	GATOTTAGAG	GGTCTAGCTO	CONCREGICE	accounting	CONCENTRAL
	CATCCCCCAG	CAACCAOCIC	GICTCCGCCO	GCCHOANINC	OCTUMENTA	CUCCANCCC	CCAGATCGAC	GCAGAGCAGG	CCCCCCAGAC	GCAOPTRACEA
6101	AMGACCCCG	DOCADENDOC	OCCOUNTON	GTASTCTATC	TRICATULET	GCANGTCTAG	CHECKINGTIGE	CATOCOCOGO	COCCAAGCGC	GCC-TCT-A1
	THETOGOGE	CCOTCOTCCO	COCOCAOCIT	CATCACATAG	AACGTAGGAA	CHITCAGATE	GCTASACGACG	פואבסבסבבב ו	OCCUPACACO	COCCAOCATA
6201	COOPTICACTO	GOOGACCCCA	TOCCATOCOG	TOCOTTANCO	CONTACTO	CATCCCCCAA	ATGTCCTANA	CCTACAGGGG	CTCTCTGAGT	ATTCCAAGAT
	CCCACTCAC	CCCCTGGGGT	ACCOUNTECC	ACCENCINGE	GCCTCCCATAT	GTACCCCCTT	TACAGCATT	GCATCTCCCC (CACACACTCA	TARGOTTETA
6301	ATOTAGGOTA	OCATCHICCA	CCCCCATATIXC	TRACIOCAC	GTANTCHTAT	ACTICOTOCG	MANAMACCAG	CACCTCCCCA (CCGAGGTTGC	TALCONCOON:
	TACATCCCAT	COTAGNAGOT	OCCCCTACO	ACCOCOCOTO	CATTAZ:ATA	TCANGCACGC	Tecentedene (CTCCAGCCCT (OCCTCCAACG	ATTACCTACCT
6401	CROCKCROCK	COGNIGACTA	TETECCTOM	GATGACATGT	GALTERATE	ATATCATICAL	ACCICITIZANO	ACCITICANCE 1	TRACERCTES	GAGACTTACT:
	GACGARACCA	OCCTACAGAT	AGACCOCACTT	CTACCCTACA	CTCAACCTAC	TATACCAACC	TOCOACCTTC	TOCANCETICG !	ACCOCAGACA	CTCTCX:ATO:

Figure 150

- 1				, acamerates	אניאניבאיני <u>ור</u>	ממנימינות	איכאנינאכזא נ	CASSICACITA (GTCCAGAGITT 1	TECTTEATER	
7, X	COCNOTICOT	GCTTCCTCCG	CATCCTCAGE						CAGGTCCCAA /	ACCAACTACT	
י כין	TOTCATACTT		TITITITIC	ACAGETERING (CACTY CAST	AACTITIONS OF	CCAGAMAGOT (CATCACATACC 1	ATCCCAAACC C	CONCINETE:	
		_	TETAGACTE	-		-			ceracecae (CTTCCOMAG	
	CCTTOCCATT	_				TEXT AGGRAA	איפאדטביבכא י	TCGCGCATAC (GAAGGCCTV":	
	GAGGTGTGGG	TCACCCCAAA	CCACAGGGAC	ACCARGACTT TOTAL	TENTENTE	CATAMOTTC	TCAGTGTCGT ANYCACAGG	COCATCORCE (CHOCHOCOMO I	AGCAAAAAGT TCGTTTTTCA	
	concert	_	_		מאכאזיימידוי	AAGACTATCT				TGCGGAAGGT	
	COCACOCCAA			CCCGCTTCCA	CTRITARICANC					ACCCCTTCC!	
	PCCC33CACC	* TCGCAACGOT	ACANTTANTO ACANTTANTO	CACCCCCCCC	ACCACCATCT TCGTGCTAGA	מכאוודדוכפם	CANCTACANC	TCCCCCACAA	ACATTICANG (CANGAAGCG" OTTCTTY:TCT:	
	GOGATOCCCT	_		_	ACCITICACITE				_	GCAAGATVAG	
	CCCTACGGGA	_	GITAMANAT	TCAAGGAGGA	TECALFICING	ANGRECECTE			-	COTTCTACT:	
	COTTOGNAGE	: CACCAATGAG			TAGCATTEC				CCTATOGCCA	TTTTTCTCC	
u	CANCETTED) CHOCKTACTC	CAGOTOTICCA	MOCCCGGTA	ATCCTUNNEG					MANAGACC	
	DOTOATOCAG				TCCCATCCAA	CCTTCCCGC	TACCITCITCOC ATTTACACAC	COCCOCAGTCA	GATCTCCGNG	ATCTCCCCC TAGAGGCGGC	
	CACTACOTO		במכנרבאוישור	Mancol Cucc	Acceptante				-	TEGOTOCOM	
-	MCTTCATOR TTCARGET	CONCORACT.							TTTCTCTGCG	AGCCACGCT	
			ACTOGATET	CCCGCCACCA	ATTOCACTAG	TYSCTATTGA				CCCAACACTC	
	CTACGCTCGO				TANCCTCCTC	ACCOATAACT	ACACCACTIT			OCCTTCTCNA	
	and nogeth	_	GIGCGCAGTA	CTCCCAGCCC	TOCACCACCT	GTACATCCTG	CACCACATTO	ACCTGACGAC		CAAGCAGAGT	
	CACCACCGAA	_		GACCGTCGCC	ACCITICACCO	CATCTAGGAC	GTOCTCCAAC	TOCACTOCTG ACCOSTICITY		CITCGICTCA	
	CASCAATTICA	A COCCOCACOCC	TCCC(ACTITY	COCTOCTOCT	CITYTACTIC	CARCINCTEGE			CASCOCACIT	ACCEPTECATE	
	CCTTANCT			CCGACCACCA	CAACATCAAG	CCCACCIAACA	GWACHIGEA		CICCCCTCAA	TRECACCING	
		_	-	AGATOTECOE	GENERALIZATET	CYSTACCTIVIA	TEMCANCATO		GAGCTOTCCA	TOCTCTOOM	
				TCTACAGGCG	CTCTTCCCCCA	GCCTCGAACT	ACTUTIVITAG	COCCITCTACC	CICGACAGGF	ACCAGACCTC	
				li d						-(
	CHECOGOGO	C OPCAGGTCAG	3 OCCOPANCETE	CTCACACATT			GOCOCOGOCT	AGATOCAGGT		Trechnodic	
	GAGGGCCCC	_		GACCTCCANA	TOCHOCUTAT	СПИСССАЮ	CCCCCCCCA	TCTAGGTCCA	CTATGGATTA	MGOTECEC	
										Tre-Private Affe	
	TOCTTOCTOR		COCCHICAT GACTTACANG AGGCCCATC CCCTACATC GATTATATA GATTACAC GACTACOCC	AGCCCCCATC	ניבנעוכעיייעונ	CACTACTATA	יין יאייריאירט	20010000		ACTOR ACTOR.	
	ACCANCENCE		GOCGERACYA COGANGETIC TCCGGCGTAG GCACACHEG CTGATACIAT GACKGGIGG CCGCCALLEG WULLCUM	TCCGGCGTAG	מממממממנענ	CTGATGCC:AT	שממממנוית	CCCCCACCC			

PMRKAdSgag MRR682

8101	NTOCATCTA	ANGCOGTOAC	GCCATGCCANT	CCCCCASANT	Nassagen	ככנאנעככניגנ	CHOCAGARA	GCCAGGGGCA	COTCOMICCO	GCGCGCGCAX;
	TACGTAGATE	TTCGCCACTO	COCCUTATION	GRANINCTICCA	TECTTOTA	GACCTAGGGG		ccorcecent	GCAGCCGTGG	CCCCCCCCC
8201	ASSASCITACI	OCTOCOCCCO	TARGETTOCTIC	_	ריגער איז אניז	CHINANCING	-	accretacar	CAACACCACC	מנכננסשונא
	TCCTCGMCCA	CONCOCOCOCO	ATCCAMOUNC	いいこうしょう	מיונט שכעיני:	CAACTAGACE	N TTACALCG	CCGNGACGCA	CTICICIOCIOC	CCGCCCACI
8301	CCTTONICT	CANACACACT	TCCACACAAT		GREETPIGACES	والاندعون فسلده وال	CHANAATCTC	Chacaconce	CCTGAGTTGP	CTTCMTASG"
	CGAACTTOCA	CTTTCTCTCA	AGCTGTCTTA	GTTAMAGUTA	CANT'AAC'IGG	כניניניאניאניני	CCTTTTAGAG	GACOTOCAGA	CCACTCAACA	GANCTATCC:
8401	GATCTCOOCC	ATGAACTOCT	CONTCINE	CHCCTNRCAGA	TCTVCCCCTC	ניטינישישבער	CACOGITICAL	GCGAGGTCGT	TOUNANTOCO	GCKTCATGAC "
	CTAGAGCCGG	TACTTGACGA	OCTAGACAAG	GARANCETET	ACAMASCRIAG	GCCCAGCGAG	CTRICCACCCC	CGCTCCAGCA	ACCITITACGE	CCCATANCTE:
8501		COTTOMORCC			TESTAGACCAC	מובבנוכדונה	GCATCGCRAG	COCCECATGAC	CACCTIGGGG	AGNITICACE "
	ACOCTETTCC	GCAACTCCGG	AGGGAGCAAG	GICHOCOCCO	ACA'RCTGGTC	CHASCIASANSC	CCTAGCCCCC	GCGCGTACTO	OTGGACGCGC	TCTAACTCG.
8601	CCACGTOCCO	GOCGAACACG	GCGTAGTITIC	GCACATACTIC	AAAGAGGTAAG	Theacastras	TGGCTGTGTG	TYCTGCCACG	AAGAAGTACA	TANCCCARP:
	CONCREGE	COCCATICAGE	CCCATCAAAG	CONTCOCCARC	TITCICCATC	AACTCCCACC	ACCGCCACAC	AAGACCCTCC	TTCTTCATOR	ATTIGGGTCCK:
		T.	EcoRiv							
8701		GATTCOFTCA	TATCCCCAA	OCCUTCANGG	CCCTCCATCS	הבדריהדאמא	GTCCACCGCG	AAGTTGAAAA	ACTORGAGIT	GCCCCCCAC
	ACCOPTICCAC	CTRACCAACT	ATAGGGGGTT	CCCCAGITICC	GCGAGGTACC	GRAGCATICITY	CAGGROCCCC	TICAACTITI	TOACCCTCAA	כככננפכנונ
8801	ACCOUNTANCE	CCICCICCAG	ANGACCOATO	AGCTCGGCGA	CACTUTCOCG	CACCTONING	TCAMAGGCTA	CAGGGGGCCTC	TICTACTICT	TCAATCTCE:
	TOCCALTICA	CCACCACCTC	TICHGCCTAC	TCGAGCCGCT	GTCACAGCGC	GTTGAGCGCG	AGTITECCAT	GTCCCCCGGAG	AAGAAGANGA	ACTTAGAGGA
									Sol	
8901	CTTCCATAG	GOCCTCCCCT	refrencer	CTGGCGGGGG	TOCARCACET	GGGACACAGC	GGCGACGACG	GCGCACCOOO	AGGCGGTCCA	CANAGEGETE
	GAAGGTATTC	CCGGAGGGGA	ADAAGAAGAA	GACCOCCOC	Αροκρατιστία	CCCTATACCG	CCATACTOC	COCOTOBOCCC	TOCCICCAGET	GTTTCGCGM
1006	GATCATCTCC	CCCCCCCCCAC	GCCGCATIGGT	CHCCCSTTSACG	Granacan	Triciacocia	GCICACITICA	AAGACGCCGC	CCONCATGIC	CCCASTTATES
	CTAOTAGAGO	OCCOCCOCTG	CCCCCTACCA	GAGCCACTGC	כנאנטנכנאצי	AGAGCACCC	CCCCTTCAACC	TTCTOCCGC	OCCUPTACAD	GCCCAATACK
9101		COCHOCCATO	COCCAGGAT	ACCCCCTAA	CGANNICATET	CARCANTROF	TOTATA	CHCCGCCGCC	GACCODACCTO	ACCCACTOO
	CARCOCCCC	CCCACCCCTAC	GCCGTCCCTA	TOCCCCCATT	CCTACCTAGA	GITGITAAGA	ACACATCCAT	GAGGCGGCGG	CICCCTGGAC	TCCCTCAGG
			poor.							
9201	g	ATCGGAAAAC	CTCTCCANA	ACCURATION	CCAGTCACAG	TOCCAAGGTA	GACTGAGCAC	CGTGGCGGGC	GOCHACOOO C	OCCOUNTCOM:
	GTAGCTOGCC	TAGCCTTTG	GAGAGCTCTT	TCCGCAGATT	GOTCAGACATC	AGCGTTCCAT	CCCACTCGTG	GCACCOCCCG	CCCICCCCCG	CCGCCAGCCC
		•					Sati			
9301	ornorneto	GCCGAACCTGC	TOCTGATGAT	GTANTTANNG	TARKETATIVE	TTAGACTGCG	CATOCTI:TAC	AGAAGCACCA	refections	Techacetrae
	CACMAGAC	COCCACCACO	ACCINCTACTA	CATTANTETC	ATCCX #: CAGA	ACTICIPOCOOC	CTACCARICTO	TCTTCGTGGT	ACAGGNACCC	AGGCCGGACG
9401	TOANTOCOCA	OOCOOTCOOC	CATTACCCCAG	OCTIONED	GACATICKEDOS	CANTICITAGE	TAGTAGLETT	CCATGAGCCT	TTCTACCOCC	ACTICITICIT
	ACTTACOCCT	COCCOMCCO	CTACCCCCTC	CGMOCNAA	CTCTACCCGC	CHECOGRAMAC	ATCATCAGAA	COTACHEDGA	AACATOCCCO	TGANGNAGA.
9501	CHECTRICATE	TTOTOCTOCA	TCTCTTCCAT	CTATICALTOC	بعدمعتهمن		CENTRACES		CCCATGCOTO	TOACCCCOAN
	CHOCOMOCOLO	AACAGGACGT	AGAGANCOTA	GATMX.GACG	כנוגבנוצנטכ	CTCAAACCTO	CATCCACCCC	OCCAGNATOR	COGENCIA	ACTROQUECT P
9601	accentant	GOCTGAAGCA	CARCTACGIC	GATICACAACO	CCACHITACITA		CHICACCTOC		ACTURANGIC	ATCCATISTICS.
	COCCACTAG	CCGACTACGT	CCCCATCCAG	ccactoriac	CCCACCCCAT	TATACCCCCAC	CACCTCCACG	CACTCCCATC	TGACCTTCAG	TAGGTACAGO

r H	Š
9701 ACAAAGOGT GGTATGGGG CGTGTTGATG GTGTAATTIC ASTTERICEAT AACTAACTAG TTAACAGTCT GGTGAGGGG CTGCGAGGG TCGTGAGGGG TCGTGAACTAG TAACAGTCT GATGAGGGG CACACTCTGG AGCCACTGG AGCCACATTS!	IDEA TOTAL STATEMENT CANADITIES ALL STATEMENT CANADITIES ALL STATEMENT CANADITIES COCCUCCUL CONTRACTOR COCCUCCUL COCCUCUL COCCUCUCUL COCCUCUL COCC
ក្នុង ក្នុង	8 1:
CHCTC	7000
60 E	900
0000	9100
SACTO SACTO	3
0 3 6 d	G SC
MCCCA	AFCCC
£ \$	į
NOTAG POSTIC	.twc.
ACT37.	ָנָי אָל יָנ
A 74.	4:C A
HEADON A	וייי
ACT TCA	<u> </u>
ACTURACY.	
TATA:	
אַנָה ה ראַכ ה	
CANC	
58	1000
0000	-1
CATA	
5 5	
MAGG	
Š	
1026	•
.	

ICACO CCOCCOCOA CCOCCATOTO	XCHCA ECCAGOTGAT GCCGGCGGCGCCCCCCCCCCCCCCCCCCCCCCCCC	NOGET CTOOCHOOTE ANGORAGE? TOUGH GRICOOCHAG TECGUROOP!
AAGHYYGG ACCAGGAGTAGT GGTATGGGGG GTTTT TIGAGGG TXFINGATIA CCATAGGGTG GTTTT FOSIV	CANCATAN GAEGATEATA TEGETAGATG TACCT GITGTATT CEGETACIAT AGAZATETAE ATGGA	THEGET CYPCAAAA TOCHCUATO TOOGOAN TOOGOAAAA ACCCC
TORGEGOOD GENEROCCE CACTEANEN CHERENCHE GENARMING ACCIONENT CHEROCOFIC CANAMONIC GOCCOCCO COCCOCCO ACCIONENTE ACTORICO ENTRE CONTRACTO CONTRACTO ACTORICO CALACOCTO CONTRACTO CO	ODDICEMBERT ARGENBUCCO GODITICORRIS GRICACITOT TICARATATA CRESIMENTA TICARAMINTO TACCTOGACA TECEMBERT GEOGGENECA COCCOCIOCO COCCOCIOCO CECEMBERTA COCCOCIOCO ARGENTATA ARGENT	OTOCHODAGO COCKECAM OTCHERANDO CRATTECHA TOTTECHEM CONCAMANO TOCHCUMEN TEGGRAGOCT CTOCKEGOT AROCKEGOT CARCAGOT CARACTEC ACAMOSTACE ACAMOSTACE ACAMOSTACE ACCORDICA AROCKEGOT TECHCOCOCO I
7 1086 A	9901 9	10001

Ü	CACCACCTCC	Real	כאפנפרייה	REAL MANUELLY MANUELLY MANUELLY MANUELLY CONTROLLY CONTROLLY MANUELLY MANUELLY CONTROLLY CONTROL	M. Malicoccia					
N 10101	AATCOTTOAC TTAOCAACTO	GCTCTAGACC CCAGATCTOG	GTOCANANGO CACUTTTICC	OTOCANANGO ACACCENTA ACCASATATAT CITECTINGI CIGATAGATA ANTICÓCANO GOTATEATO COOLOCATA CACATATACO CONTOCATA COCECETAS CACATACATA CACATACATA CACATACATA CACATACAT	AGCGBGTACT TCGCCCGTGA	CTRECGROOT	CTCCTFTCATA	AATTCOCAAG TTAAGCGTTC	CONTRACTOR COCACCACCT CCATAGTACC GCCTGCTGT	CCCTGCTGF
10201 G	CCCAAGCTCG	CCCUTATCCG	OCCOPICATO COCCAGGOST	GTRATECATE CACTAGGTAC	CCCANTRACKS GCCAATCACTO	GRIANTECANG CONTINUEURC CEGETHING ANICHANTIG TOCOACONA GRENOGRAD CACTAGETA GEOVARIANIO GRENOGRAGO THEATHCEAC ACCENTEGAT CHETTAGECCE	AAKCCAAATIG	ACCCTOCAGT	CHGTTGCCCC	CTCACCAGGA
10301 T	THOCETTEC AACCGAAGG	Trchococ Mooreese	CCCCCCACCA	GOCOGCTICA GCGCTAGCTT TTTTGACCAC TAXCCGCGG CAXCGTAGGC GGTTAGGCTG GAMACGAAA CCGCCGACGA CGCGATCGAA AAAACCGATG ACCGGGGGG GTCGCATTCG CCAATCCGAC CTTTGGCTTT	TTTTTTTCAC AAAACCGTTG	TRACCOCACA ACCOSCOCOC	CHACCTANGC	CCANTCCOAC	CTTICGCTT	CGTAATTCAU
10401 G	CONOCONOO		CCCANTAAAA	FORACCOGA GEOTTATTIT CCAGGOTTO AGRICACOGA CCCCOGATIC GALLICIA CULLICAAL ACATCOGCTO CTCAGAGCT GALLICAAL GALLICAAL GALLICAAL TAACCCCCTO GARGOCCAM CTCAGAGCT GACGGCTO	ACTICIOCOCOCOTA TCMCCGCCCT	CCCCCGGTTC	CTCAGAGCCT	COCCOOCCED	ACCCCCCTTG	CCCCANGI
10501 C	CACCCCOTCA			TOCANGUECE CUCTUCEAAA TIECTECEGA AACAGGAGG AGCECETITT TIGETITICE CAGANGEAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA	AACAMOCANO THORICCCTGC	THECHECISA ARCHAGONE AGCECCTITIT THOUTTHEE CAGNICATE CONTROLLORS ANGRAGACET THETECETIC TEORGIAMA ANGRAVAGO OPETACOTIA GEORGIACOC	AACGAAAAGG	CAGANGCATC	CCCACCACCC GCCACCACCCC	COTCTACGO
10601 C	CCCCCTCCTC	AGCAGGGGCA TCGTCGCCGT		ADAGCAMING CNOCGRENS CNICENSMERS ACCITECTIFIC CUITEMAND OCCUPANTICS TO STRUCTURE GOOGETICS COORDINATED THE STRUCTURE GOOGETICS COORDINATED THE STRUCTURE GOOGETICS COORDINATED THE STRUCTURE COORDINATED	CATCACACACAC	CAGCGACAGA CATGCAGARC ACCTCCTT CCTCCTACG GAGTCCTCC GTCGCCGTC GTAGCATAGC GCAGTCCTCC	GENGENTAGE	GCAGTCCTCC	CCGCTGTAGG	CCCTGTAGG CCCCAACTYC
10701 0	COCCACCACA	TOSTUATTAC	CHIGGGGGG	GAACCCCCGC GOCACCGGC CCGCACTAC CATGACTTGG AGAINGGGCGA GAGLUIGACU COCCTANANA CTTGAGGGACG CCACGGCCCG GGCCTGATG CACCTGAACC TCCTCCCGCT CCGGACGCC GCCGAACCTC	CCCCCACTAC	GOCYCCGGC CCCCCCTAC CTGACTTCG ARTHGGCCGA GGGCTUGGG CUCCTAGAGGCCG GCCGATCCTC CCCCGGACCGC GCCGATCCTC TCCTCCCGCT CCCGGACCGC GCCGATCCTC	ACTECCOCT	CCCGGACCCC	OCCOARCETE ACCOARCETE	GCGGGACAC*:
10801	TRANCOCCAC ACTCCCCCTO		CCANGGOTCC AGCTGAAGCG GGTTCCCACG TCGACTTCTC	CCANGODICE ACCHGANGCO TONTACGCOT GAGGCOTAGG TOCCAGATA GAACCAGTT COCUMACTOCAGAGAA GAGCTIGGCOC COCTAGAGACAA GAGCTIGGCOC COCTAGACACAGAAA GAGCTIGGCOC COTACAGACAAA GAGCTIGGCOC CAGACACAAAAAAAAAAAAAAAAAAAAAAAAAAA	CHCCGCATGC	TOATACCCTT CACCCCTACC TCCCCCTA GAACCTITT COLUMNICUM ACCOMMENS ACTATCCCCA CTCCCCATIC ACCACCCCCAC CTTCCACCAA CCGCTGCCCC TCCCTCCCT	CTTCCACAA	COCUMPCEUCE COCCOCOCOCOCOCOCOCOCOCOCOCOCOCOCOCOCO	TCCCTCTCCT	TECETETECT COORTECTE TOACCCCOACCC
10901 A	ATOCOCOCATC TACCCCCTAG		CGCAAGGCGC	GAAAGHECA COCAAGGGG GAGCHGCAR ATRACLTHAN THELSAKAN THE TOTAGGGGG TOCHCGGA ACTEGGGGG COCATTGGG CHTICAAGT GCGTCCCGGG ACTEGAGGGGG TACAGGGGGGG TACAGGGGGGG TACAGGGGGGGGGG	TACCOCACTT	AGCGCTCGCC	AArgacococ	TCCTCCTGAA	ACTCOOOCTO	COCOUNTOGC

ACTAACCICA TACHARCAGA COOTGANICA GOAGATDAC TITCAAAAA CCITTAACAA COCHOGNOS OFFOCTATA GRAFICATIVE ATCHTICARA CTTTFFANGE GEOCTOGAGE ANACCEAIA TYCCIARRICE CCCCACCICG TITIOGRITT ATCGITCGRG **CTCATOGUCC AGCIUTICCT TATAGITICNG CACAARTAFA ACAARGACH: ATTCADART GCCCTAA ACATAGITAGA GOUGAGGGU COCTOGUTHU** GAOTACOGOG TOCACAAGGA ATATCAGGIC GIGIUCATUC TGTTGUTCIG TANGTUCCAA GOUGACAAT TGTATCATOT COGGUTUCEG GOGACCGACG AANGTITIT CCAAATTICTI CCTCTANTIG מושכתכנשב משכמשכומות בכתדומשבה תתמידונהוכד שרבתדותה OUTGENEUGA TECCAMENCE GOOCGENECT CENECGATAT CETGACTIMES TAGACACET GAAACATECS CACGTOOCHO CCGCCOACCT ACCCENICACIO COCOCOCOCA GCGCGCGCGT CONTRACTOR CCTAATCAGG CCACOTOCOF 11001 11101 11201

11301	TECATITEAT	-	CAGAITTATAG	カンプレス・ファン・ファン・ファン・ファン・ファン・ファン・ファン・ファン・ファン・ファン	CHTYNYTHG		ACAMOSTOR	CCCCATCAAC	TATTCCATGC	TTAGCCTOTA
	ADCTANACTA	TITICTACCAC	GICTUCATATE	ACC:ACS STYCE	CLAST TECTOR	TI TATACLICAC	Terminian	CCCANTACTED	ATANGGTACG	ANTICACACITY
11401	CAACTITAC		TATACCATAC	CCCTTACGIT	CULTATALACA	ACTIACITAAA	CATCCACCACG	TTCTACATOC	CCATCCCCCT	CAAGETTECT .
	CTTCAMANO	COCCCCHCT	ATATOSTATO	GCCAATGCAA	(איזידאדריתיה	TITLE	CTARRITECTO	AAGATKITACO	COTACCOCOA	CTTCACCV 1
11501	ACCTICACCO	ACCACCTOGG	CGITTATCCC	MCGACTICCA	TICK NAME	CARTGAIRCORD	MICCOCOCO A	OCCIAGOTICAG	CGACCCCCTAG	CTISATISCACA
	TOOMCTOOC	TOCTOGACCC	CCAAATARCO	TRICICOCGE	NOTICETICS	GCM:TCCCCAC	ידייהפכינינים	COCTCGAGTC	GCTOGCCACTC	GACTACORUS
11601	OCCITOCINAD	GOCCCTGGCT	GGCATGRACA	CCCCCCATAG	NUMBERCANG	TCCTACTTTG	ACGCGGGCCC	TGACCTGCGC	TGGGCCCCCAA	GCCCAACTCGT
	CCCACCOTTIC	CCGGGACCGA	CCGITICCCGT	CACCINETATE	TURCOCT.	ACCIATICAAAC	TOCACCCACG	ACTOGACOCO	ACCCCCCCCTT	coxcrococ:
11701	CCTGGAGGCA	actobacco	GACCTOCCCT	CATC TOTAL COLA	בבנננינונטנטינ	CTGGCAACCT	CONCRECENT	GACCINATATE	ACGAGGACOA	TONGTACGAG
	CONCETEDOS	CCACCCCCCC	CHOCACCCCA	CCCCCACCGT	CONTRACTION	GACCGTTGCA	OCCCCCCCAC	CTCCTTATAC	recreeter	ACTICATOCTIC
									Psil	
11801	CCAGAGGACG	OCCAGTACTA	AGCGCTCATC	TITICITICATICA	CATCATGCAA	GALLICEAACTAG	ACCERACIAST	0000000000	CHOCAGADCC	AGCCGTCCC?:
	GOTCHCCTGC	COCTCATGAT	TCGCCACTAC	AMGACTAGT	CTACTACGT	CHOCHEREC	TOGGCCCATCA	CCCCCCCCCCC	GACGICTCOG	TCGGCAGGC
11901	CCTTAACTCC	ACCORDCGACT	GOCTOCCADOT	CATGGACCISC	ATCATGTOCC	PRACTYACORO	CAATCCTFAC	acontocoac	AGCAGCCGCA	GGCC/MCCP1
	CCANTTOACC	TOCCTOCTGA	CCCCCCCTCCA	GTACCTIGGCG	TAGTACAGEG	ACTGACGCCC	CTTAGGM:TQ	CGCANGGCCG	TCGTCGGCGT	CCGCTTGCC.
							P. Carre			
12001	CTCTCCGCAA	TTCTOGARGE	OCHOCICCO	OCCCOCCCA	ACCCCACTCA	CRACANOCTC	CHOOCOATUS	TANACCOCCT	GCCCGAAAAC	AGGCCATC
	GAGAGGCGTT	AAGACCTTCG	CCACCAGGGC	COCCCCCCTT	TOCOGRACOT	ACTOTACCAC	GACCACTAGC	ATTICCCCGA	CCCCCTITICS	TCCCOOTAG
12101	GGCCCGACGA	GOCCOGCCTO	GTCTACGACG	CHICHGETTCA	ACACATORET	CCTTACAACA	GCGCCANCET	GCAGACCAAC	CTOSACCOOC	TOTOGOGGA
	CCOOCHOCT	CCGGCCGGAC	CAGATGCTGC	GCCACGANGT	COCCICACCGA	CCANTICITALIT	CICCUITICCA	ceretoerro	GACCTGGCCG	ACCACCCCC"
12201	TOTOCOCOAG	OCCUROCOC	AGCGTGAACG	CACCACAGCAG	CARGOCANIC	TOCOCTOCAT	GGTTGCACTA	AACOCCTICC	TGAGFACACA	OCCCOCCAM.
	ACACGCCCTC	COCCACCOCO	TOCACTOOC	acacancan	GTCCCGTTGG	ACCECTAGGTA	CCAACCTCIAT	TTGCGGAAGG	ACTCATICTUT	COCCIONATI
12301	OTOCCOCOO	GACAGGAGGA	CTACACCAAC	TTRIFFGAGCG	CACTGCGACT	AATKISTIGACT	CANGACACTOC	AAACTGAGGT	OTACCACTOR	GOTTOTAGACT
! !	CACOGCGCCC	CTGTCCTCCT	CATOTOCTTO	AMCACTOGO	GTCACCCCTA	TTACCACTGA	CTCTGTCGCG	TTTCACTCCA	CATOGICAGA	CCCCCCTCTGA
)š.d.	-						
12401	AFFFFFFCCA	GACCAGTAGA	CAAGRECTICE	NGACCGTABA	CCTMARCCAR	ACTITICAMA	ACTITICCAMAG	CCTCTCCCCC	OTRICOGETE	CCACAGGGGA
	TAMANAGGT	CTGGTCATCT	GFTCCGGACG	TUTOSCATIT	GGACTCGGTC	CCANNOTITY	THANGETOCC	CCACACCCCC	CACOCCCGAG	OCHCICCOSCT
12501	CCCCCCCCACC	GIGICIANCE	TOCHGACOCC	CAACTORCIAC	CTGTTTT Trac	TYRCTAATAGE	GUNCHINIAGG	GACACTOGCA	OCCUTETICCO	GGACACATAC
	0000000000	CACAGATCGA	ACGACTGCCO	OFTGAGCGCG	GACAACGACG	ACGATTATCG	CTATALANCTESC	CTCTCACCGT	CGCACAGGGC	CCTOTOTATO
12601	CTAGGTCACT	TOCTOACACT	OTACCGCGNG	CCCATAGNTC	Arschalater	CHALLACTAT	ACTITICCARG	AGATTACAAD	TOTCAGCCGC	OCCECTAGONIC
	GATECAGTGA			COGTATCCAG	TCCCCCTACA	CCTCACATC	TENNACOTEC	TCTAATGTTC	ACAGTCOOCG	COCCOVCCCCO
								_ 3	Presi	
12701	ACCACACAC	COCCACCTO	CHARCACTC	TANACTACET	CATTEMETANG	CHACHACAGA	ANATOCOCTO	OFFICEACAGE	TTAMCACC	ACCACICAGIT
	TECTECTOR	CCCOTCOORC	CTCCOTTYEST	ATTICATION	ההאכיוואניויה	CUCACCTUT	TUTACKARAG	CAACGTGTCA	ANTHOROGO	PCCTCCTXXX.
12801	CATTITIOCOC	TACOTICACK	MCACCTOTIONS	CCTTANCCTG	ATTACCACTE	CH KITAACINIC	CARCATICALO	CTURGACATGA	נכטכטכטטט	CATCSCALCTE
) 	OTAMACGCO		PUTTICACTO	REMATTRIBAC	TACTATA TAC	CCCATTRACGG	כשבנאניאינוגאנ	GACKTINGTACT	GACCACCOCTT	GTACCTTOOT:

Figure 15H

PHRKAd54Ag NERGR2

12901	CCCTACATAC	CCTCAAACCT	CCCCTTTATC	AACCURICTAA	TYX:ACTT ACTT:ATTGA	CECATICISTICS CO	הריארנארנא האקלאיניאיד	ACCCCCMOTA	TTTCACCAAT	CCCATCTTCA CTZTACAACT
13003	ACCCRCACTO TCGCCSTGAC	OCTACCOCCC CGATGCCGCO	CCTCATTTACT	ACACCTATATA: TTTTT RECUCE	ATTECACATE: TAAGRITECAC	CONTRACTA			GACATAGACG	ACACACACAT TOTAL TOTAL TOTAL CALLAR
13101	TICCCCGCA	CCCCAGACTC		_	GAPTARAGAG MXXXXXXXX	MRRICART	-,		GOCCAAGCAG	CTIGTCCGAT
	AAGGGGGGTT	0000101000	ACCATCTICAA	CGTTGTCGCG	CPCCTTYTTE	Truckting	CULTITICETT	TCGMGCCGT	ccoornear	GANCAOGCT'.
13201	CTAGGCGCTO	COCCCCOCO		OTCADATOCT AGTACCCCAT	TTCCANITT GATACETTCT	GATACECT	CTTACCARCA	CTCCCACCAC	CCCCCCCCCC	CTOCTORGO:
		-	Pica and a second	ICONTANCOIN I	WAS I COM				מינימייניינייניינייניינייניינייניינייניי	הארכה
13301	ACCACCACA	CCTAMCAAC		TEGETISCING ANCOCCAGOS	CGANAMANC	ביומכביובנימ	CATTROCCA	CAACGOGATA	CACAGCCTAG	TOGACANIAT
13401	CACATCACC	AAGACGTACG TTCTGCATGC		CARROACTE	CCAGACTUC				oftenedono charcoccc	TCTOGFTGTCP1 AGACCACACT
13501	CACCAGCTAC	ACTCGGCAGA TGAGCCGTCT	CCACACTCACC	CAGGACCTAA	TRESPACIFING ACCURACYTO	PRECANCEES ACCOTTRACE	TTTGCTCACC NANCTCCTTG		CCACCCCTCT	ATCTITITAAA TACAAAATTT
13601	ANNAMAAA	GCATGATGCA	AAATAAAAA	CTCACCAAGG	CCATINGCACC	GAGCTTTRATE	TETCTTGTAT AAAGAACATA	TCCCCTTANT	ATGCGGCGCG	COCCICTACTA
13701	TOAGGAAGOT	CCTCCTCCCT OGAGGAGGGA Npml	ectnearno ggatteenere	TOTOGOTOANC ACACCACTCG	CUCCUCUCAG	TRANSCOCIONS ACCOCCOCIO	CCACCCAAGA	CCCTTCGATG		CCCCCCTTT GGCCGCCA
13801	GACCACCAC	CCATCCACCC	GCCTACCONS	CCCTCTTTGT	CCATCCCTTA CCTAGGCAAT	CTCTCAGTIG	GCACCCTTAT CCTGGGGATA	TCOACACCAC ACCTGTCCTC	CCGTUTGTAC	CTGGTGCACA
13901	ACAAGTCAAC TOTTCAGTTG	GGATGTGGCA	TCCCTFAACT AGGGACTTOA	ACCMANGGA TROTCTTGCT	CCACACCAAC	TTTCTCACCA	CCCAGTANGT	MACMATGAC	TACAGCCCOO ATCTCGGGCC	GGCAGGCAAG CCCTCCGFF
14001	CACACACACC	ATCANTCT10 TAGTTAGAAC	ACCACCOCTC TGCTCCCCAG	GCACTRAPARC COTTGACCCCG	CCCICTACCTICA	AMCCATCCT	CCATACCAAC	ATGCCAAATG TACGGTTTAC	TOANCOAGTT	CARCHITACE GTACAAATCA
14101	ANTARGTTTA TTATTCAAAT	AGGCGCCCA	GATOCTOTOS CTRCCACAGO	COCTTOCCTA	CTAAGGACAA GATTCCTV7TT Pwd	TY AGTECACY TO	CTCAAATACG	AGTOCOSTORIA TCACCOCACCT	OTTCACGCTG CAAGTGCGAC	CCCCANTACA
14201	ACTACTCCGA TGATGAGGCT	GACCATCACC	ATM:ACCTTA FATCT::CAAT	TGAACAACIC ACTICITICO	GATCHTHAIAG CTAGEAC(TC	CACTACTTCA	AAGTGTGCAG	ACAGNACOCO TOTETTOCCC	GTICTOGAAA	CACCACATOGG
14301	CCATTICAAA	CACACCCCCA	ACTTCAGACT TGAAGTCTGA	CCCCTAAN; Th	CCTOTTONIA GRANINGTING	CACAACACTA	CHANCELAT	TATACARACO	ANDCETTECH TTCCCANCOT	TCCAGACATU AXSTCTGTAG
14401	ATTTOCTCC TAVANCGACG	CHREATUCOS	CCACCTRAAG	ACCCACACAC TOXATIGINOS	התכדינאת:AA מממערניהד	CTTCTTCTCCCC	APECTYFANGE TAGGETTEG	CCCTTCCCAA (CCAGGAGGGC	TTTAGGATCA ANTECTAGT

Figure 15I

14501	CCTACGATUA	TCTGGAGGGF	CCATTESTANG	CCCCACHCTT	CHANTIGRAC	CATCANCICANC	CGAGCTTGAA	AGATGACACC	CTTGTCCCGC	ממפניוניטינוני סמפניוניטינוני
14601	AGGCGGCCAGC TCCCCCGTCG		OCA! RIPRICAL CUMINDOCYRIG	CCTICICATIO	TRYCAACTICKS; ACKETTYNCY		AATGCACCC	GACCTCCTGT	TRAACCIATICA	TOCCATTOTAL ACCOTANGED
14701	GOCGACACCT	TTGCCACACO AACOGTGTGC	COCTCACCACACACACACACACACACACACACACACACAC	AMEDIATECTOS TTCGCGCGAC	AGRECT TRANSE	Managanan Managan Keta	GCTV ATCOR TTC CTACGOC(TATG	CCCCTGCGCA	ACCCGAGOTC TOBOCTCCAG	CACAMACCTA CACATACGAN Mand
14801	AGANGAAACC TCTTCTTTGG Kpm	CCACTAGITT	CCCCTTACAG	APPIACAGEAA TCCTGTCGTT	GAAACGCAGT	TACAACCTAA ATUTTCGATT	TAMECANTOA	CAGCACCTTC GTCGTGGAAG	ACCCAGTACC TOGGTCATOG	COTCUACCAT
14901	CCTTOCATAC	AACTACOGCO TTGATGCCGC	ACCCTC ACAC	COCHANCEGE	TEATERINGE AGENCIENCE	Trictititicae Actianancitis	TCCTOACCTA ARSACTIXAT	ACCTGCGGCT TOGACGCCGA	CCCAGCAGCT	CTACTCECTC: GATGACCARIC
15001	TTGCCAGACA	TOATOCAAGA	CCCCCTCACC	TTCCGCTCCA	מפונפיניאמאד מכוזכנטטזכדא	CARCAACTIT	CCGSTRACTING	GCGCCGAGCF	OTTOCCCGTO CAACOOGCAC	CACTCCANGA GTGAGGTTCT Asd
15101	OCTICTACIA COARGATGIT ARCI	COACCAGGCC	GPCFACTCCC CAGATGACOS	AACTCATCCG	CCAGTTTACC GGTCAAATGG	TETETGACEE AGAGAETGGG	ACTIVITICAA TOCACAAGIT	TCGCTTTCCC AGCGAAAGG	GACAACCAGA CTCTTGGTCT	THIRGGERY ANACCUCY
15201	CCCCCCNGCC	CCCACCATCA	CCACCGTCAG	TGAAAACOTT ACTTTTGCAA	CCFRICTCTCA GCACGAGAGT	CAGATCACC	CACCCTACC	CTGCGCACACA	CCATCOCACC CCTAGCCTCC	AGTCCAGCUA TCAGCTCGCT
15301	CACTGGTAAT	CPOACOCCAG	ACCCCCCCCCCCTCCC	FUCCCCTACG ACCOCCATGC	TTTACAAGGC AAATGTTCCG	CCIVERACCOTAL	OTCTCCCCCC CAGAGCCGCG	GCGTCCTATC	CHCCGCCTCACT	TTTTCACCAA AAACTICITT
15401	COTACAGOTA	CCTTATATCO	CCCAGCAATA	ACACAIXBETG TOTGTCCGAC	TRANCTIOCOC CCCOGACICIO	TTCCCANGCA AACGCTTC: T	AGATGITIOO	COCCCCATIC	AAGCGCTCCG	ACCAACACCO
15501	ACTOCICCTO TCACCCCCCCC	COCOOOCACT	ACCOCOTOTC TGGCGCCCCG	CHARACTEC	CACAAACTECTE	CCCGCACTOC	GCGCACCACC	GTCGNTGNCG	CCATCOACOC	GOTOSTACAS CCACCASCT
15601	CACCOCCCC	ACTACAGGCC TGATGTGCGG	CACCICCHOCA	CCAGAGATCA GEACACAGGE	CACTOGACGC	COCCATTICAG	ACT:GTG/TTC TCT:CACCACG	GETACADECETO CIGCETTOGGGE	GCGCTATGCT CGCGATACGA	ANATONGA
15701	GACCOCCACAC	90	COTCACCACC	GCCGCCGACC	CHART ACTION OF THE CONTROL CHART ACT ACT ACT ACT ACT ACT ACT ACT ACT AC	GCCCAACTACA	מככטכטכטכס	CCTGCTTAAC	COCOCACOTO GCGCOTOCAG	GCACCTARICES
15801	ACCCCCCCCC	ATGCCCCGC TACGCCCGGC	CTCCIAAGGCT	GCGGCGCCCA	ATROPCACTG TAACAGINIAC	TERCESCECIO ACTADORAÇÃO	OTCCAGGCGA CAGGTCCGCT	CCACCCCCCC	CCGCAGCAGC	CCCGCCCATT
12801	AGRECTATGA PCACGATACT	-	CARRENCAAC	GREATANGO CAEATANGO	NCCCGCGCTGAG	CCANTOTECG	כדוגכעכהדטכ מאכפנהניאפס	CCGTGCGCAC	CCCCCCCCC	COCAACTACA
16001	TTOCANGAAA	ANACTACTTA TTTCATGAAT	GACTEGENCE	CAACATACAT	PCCAGGGGGG	CCCCCCCCCC	ACCAMPETAT	GTCCAAACGC	AAAATCAAAG TTTTAGTTTC	ANGAGATICE TTCTCTACGA

figure 15J

COCTIDORCE SOFANCTATE TEGRACIATO GANGACATCA ACTITICORIC PCIDICCOCO COACAGOCT CICOCOCOGIA CATOCOTAGA TAGONATA ACANCOTAGA CITOCOTAGA HOAAACACAGA GEOCOGIACA GOOCOGIAGA GACCOTICATA ACANCOTAGA CITOCOTAGA HOAAACACAGA ACAGOCAGA COAGAGACAA GACCOTAGA CATOCATATA

17501

5

16101	CCAGGTCATC	- :	TCTATOOCC	CCCDANGANG GANGANICACS	CANTANTANT	ATTACAACC	CTCAAAACTA	ANGCODOTCA	AAAAAAAAA	GNANGATERAT
	COTCLACIAL	רמנשמנות	MUNITURE	MUNICIPAL GOSTILLIC	CIN WAR		1 50.41.7.8.	Sadi		
10091					.,		Dag and and	CHALL MANAGEMENT	BABACTERITE	. ALLEGE COLOR
	CTACTACTIO	AACTGCTGCT	CCACCTTCAC	_	CARCOCCOCCOCC	CYCTRICCI'AT		CAGCTUCCA	TTTTGCACA	AACCCTGC*
16301	GCACCACCGT	AGRETTTACO	CCCORTCAGE	GCTCCACCCG	CACITACAAD	COCCENSTANCE	ATCAGGTGTA	COCCACTA	GACCTOCTTO	NOCAGGICAN
	COTOGTOCCA	TCAGANATGC	RESCUACTOS	CONTOTACO	GTCKATGTTC	CHICACATAC	TACTCCACAT	OCCOCHOCHO	CTOGACGAAC	TCGTCCGGTT
16401	COARCACCTC	_		REGECATANG		المسلمين والم	ההאכפאסיאכ	ANCECANCAC	CTAGCCTAAA	OCCCSTANCA
	OCTCGCGA0	CCCCTCAAAC	COATCCCTTT	COCCCTAITE	CTGTACGACC	GCANTORCHA	ccnscrccos	ricocriona	GATCOGATIT	COCCATTGT
16501	CTUCAGEAGG	•			ACCICCIONET	AAACTYTECAG	TCTGGTGACT	TOOCACCCAC	COTOCAGCTO	ATKATTAKICCA
	GACGICOTEC	ACGACOCOCO	CCAACOTOGC	AGG:TTCTTT	Tenegreenth	THEOREGETE	AGACCACTGA	ACCGTGGGTG	GCACOTICGAL	TACCATVAGGT
16601	AGCOCCAGCG	NCTOGRADAT		ANATIGACCGF	GGAACCTRAGO	CHTAGARACCCG	AGGICCOCOF	GCGCCCAATC	ANGEAGGTOG	CCCCGGGACT
	TCGCGGTCGC	TONCCTICTA	CAGAACCTTT	TTACTORICA	ככידומכאנייב	CACCTCGGGC	TECHNOCOCA	COCCOOLITAG	TTCOTCCACC	OCCCCCTGA
16701	OCCUTOCAG	ACCOPIODACO	TTCAGATACC	CACTACCALT	AGCACCANTA	TRACCACCAC	CACMBAGGGC	ATOCAGACAC	AMCGICCCC	GOTTOCCTCA
	CCCCCACOTC	TOCCACCTOC	MOTCTATOC	CTCATOCITCA	TCCTCTCAT	AACGGTGGCG	Grencicces	TACCTOTOTO	THYOCAGGG	CCAACGGAGT
16801	0000000000	ATOCCOCOOP	OCAGGCGGTC	actionacco	CONCENSAC	CECTACCARAG	GITTECALANCTAG	ACCCGTGGAT	GTTTCGCGTT	TCAGCCCCCC
	COCCACCOCC	TACOOCOCCA	COTCCCCCAG	CGACGCCCCC	CCACTTCTG	GAGATACCITC	CACGTFICACC	TOGGCACCTA	CAAAGCGCAA	ACTECACACA
16901	000000000	CCCFTCCAGG	ADICTACOCCO	CCCCCAGCGC	GCTALTISECE	GANTATRECE	TACATCCTTC	CATTOCOCCT	ACCCCCGGCF	ATCGTCGCT
	CCCCCCCCCCCCC	GOCARGETEC	TEATGCCCC	GOCCOTCGCG	CCATCACCCC	CTTATACGUG	ATCTAGGAAG	GTAACGCGGA	TOGGGGCCCGA	TAGCACCGAT
17001	CACCTACCOC	CCCAGNAGAC	CARCANCTAC	CCGACGCCGA	ACCALCACTO	מאכנוכפנכם	CCCATATATACGC	CETCGCCAGC	CCGTOCTAGE	CCCGATTTCC
	GTOCATCCCC	OCCUPATION (CTCCTTCATO	GOCTICCOCCT	TOSTOSTOAC	CTTORGEGGC	OCCORCYOCG	GCAGCGGTCG	GOCACGACCO	GOCCTNANG
17101	CTCCCCAGG	TOOCTCUCGA	ACTINGTOCACT	ACCURAGICA	TRECONCAGE	CKECTACCAC	CCCAGGATATEG	TTTAMAGCC	CONCINCIO	OTTICITIOCAG
	CACOCOTCCC	ACCCAGCGCT	recreesee	TRACACCACG	ACCEPTION	CCCCATGGTG	GCCTCCTAGC	AAATTTTCCC	CCAGAAACAC	CAAGAACGT
										Sphi
17201	ATATOCCCT	CACCTUCCOC	CICCOTTICC	COSTACCOCC	ATTCCCARGGA	AGANTOCACC	GTAGGAGGGG	CATGGCCGGC	CACOOCCIOA	COCCURRA
	TATACCOCCA	CTOGACGGCG	GAGGCANAGG	GCCACGOCCC	TANCTOCT	Transmin	CATCCTCCC	GTACCOGCCG	OTGCCCGCACT.	OCCCCCCTA
	Ē				Hels.					
17301	aconconac	CACCACCOOC	GCCCCCCCCC	GHISCACCOF	CCA; ATTACOSCG	GCGGTATCCT	OCCCC-FCCTT	ATTCCACTOA	1CCCCCCCCCCC	GATTGGCGCC
	COCHOCHOSE	orcorocco	ככפככפנפכם	CAGCGTGGCA	OCCITACOCYC	CONCATANKA	COCOSTACCAA	TARGETERCT	AGCGGCGCCG	CTAACCUCGI
17401	GTUCCCOGNA	TTOCATCOF	OCCUPACAO	GCCCAGAGAC	ACTRIATTANA	AACAAGTTAC	ATCTACAAAA	ATCARATA	ANAGECTOGA	CTCTCACCCT
	CACOOCCET	_		_	TCACTAATT	THETHEMES	TACACCTITIT	TASTITUTE	TTTCAGACCT	GAGAGTCCCGA

Figure 15K

	Ecoffy									
17601	ACCAG	CANTATGAGE	מיונאינטכבו	TCARTTERDO	CACGUAGE					GCARITAARO
	ACCCOTOGIC OF	OFFATACTICS	CCACCGCCCA	ACTICACTOC	פאהנדואנאנדו	TYSCCGTAAT	TITTAMACC	ANGCTOCCNA '		CONCONTOCO
17701	CTOGAACAGC AG	OCACAGREC	AGATTACTICAGE	CHATANTTRO	AAAGAGAAAA	ATTRICKARCA	AAAAAATAA		CCTCTGGCAT	TAGCTGCTCT:
		restorces	TETACGACTE	CCTATTICAAC	TTTY:TI,YETTT HONOR	TAAAAATTAT	TTTCCACCAT CTACCGGACC		GCAGACCGFA	ATCGCCCCN:
1780)	CHOCACCTOO CC	CAACCAGGC	ACTGCAAAAT	AACATTAACA	GTAVATTICA	Trecedence	CCCTTAGAGO	ACCETCCACE	COCCEPCOAD	ACASTISTOP .
	U	STREETCC	TCACCTTITA	TICTAATIGE	CATTURARIT		GENERALTIC	TCGGAGGTGG	CCOCCACCTC	TOTCACAG7 ::
17901	•	CCCCAAAG	COMICCOCKE	CCGACARTA	ACANACTECTS	TTY: VICTORY NAMED	TASACCACIC	Tecenegrae	GACCACCAC	TAMBETANT:
ı	_	ACCOCHITIC	GCAGGCGCGG	GACTOTOCOCT	TUTTICAGAC	CACTOCGETT	ATCTGCTCGG	AGGGAGCATG	crecrecerd	ATTECTIC:
18001	CCTOCCCACC AC	SCCENTCCCA	PCGCCCCA7	GCCTACTOGA	CHTS-TUTOPCC	איינאניאניעכע	COTAACCCTC		CCCCCCCCA	CACTICABOOM
	CCACCGCOTCC TO	NGGCAGGT	AGCGCGGGTA	AGCCCCCCTA . CCCATYXX;CT	כאכנואכנוכנופ	TCCTCTTTTGG	GCATTGCGAC	CTGGACGGAG	GGGGCGGC7	Griscorcon:
18101	AAACCTOTOC TO	MOCCAGOCCC	CACCOCCOTT	GITGIAACCC	GECCTARGCG	COCCITACOTO	כפכנפנפנפנם	CCAGCIGATCC	OCCUNTEGITO	CUCCCOTAG
	_	ACCOUNTECTOOD		CAACATIVATO	CAGGATCAGC		CCCACCCCCCC	дотсиссло о	COCTAGCAAC	GCC(A)CCATC
18201	_	CTOCCANGO	ACACTOAACA	CCATCCTCCC	TCTGGGGGTG	CANTCECTYA	AGCCCCANG		TARCTAACOF	Greenatorn
	_	SACCOUNTED	TOTOMCTION	CGTAGCACCC	AGACCCCCAC	GITTACCCCACT	TCGCGGCTTCC	TACCAMGACT	ATCGATTGCA	CACICATACA I
18301	TOTCATOTAT OC	SCORCCATOT	COCCOCCAGA	ACARCTECTO	ACCICCCIACIA	COCCOCCTT	CCAAGATTAGC		AFCATOCCCC	ACTOCTOTA
	_	COCAGGTACA	GCGGCGGTCT	CCTCGACGAC	1conconac	CCCCCCCCCAAA	COTTUTACE	ATGGGGAAGC	TACTACOOCO	TCACCAGA: r
18401	CATOCACATE TO	PCGGGCCAGG	ACCCCTCGGA	GTACCTGACK	CCCCASCTGG	TENSTINE	CCOCOCCYCC		TCAGCCTGAA	TANCAROTIT
	_	ACCCOGNICC	TOCOGAGCCT	CATGGACTCG	משמנונינישענב	ACCTUANNO	OCCUCODIOC	CTCTGCATGA	AGICGGACTT	AFTICTICAM
18501	_	CONTOCOCO	TACCCACGAC	GTGACCACAG	ACCIMINATION	COCELLICACO	Chriciantica		CCCTGAGGAT	ACTICICITALIT
	_	SCCACCOCOO	ATGCOTGCTG	CACTOSOTOTIC	TONCCACAGG	COCAMACTIC	GACCCCAAGT	ACCORACACCT	GCCACTCCTA	TCACCCATGA
18601	COTACAAGGC GC	ACCUTICACC	CTAGCTVTGG	CTCATANCCS	TOTOCTOGAC	ATGRICTICCA	COTACTITICA		GIGCTGGACA	GOOTCCTA.
	~	SOCCAROTOG	CATCGACACC	CACTATICKOC	ACACGACCTG	TACCGARGGT	GCATGAAACT		CACCACCTOF	CCCCGGGAN
18701		MCFCTOGCA	CTCCCTACAA	COCCUMBER	CCCAAGRATIG	CCCCAAATCC	THACGAATGG		CTACTOCTOT	TGAAATAAN
	_	NTGAGACCOT	GACGGATCTT	GCGGGACCTA	COCHICEAC	COCCETTAGG	ANCOCTTACC		GATCACGAGA	ACTITATIVE:
18801	CTNGAAGAAG AG	NOCACCATICA	CACCANGAC	GAAGTACACT	MACAMACTEA	GUNGUNAAA	ACTCACCITAT		OCCUPATION	CCTATANATA
	GATCTTCTIC TO	ICCTOCTACT	GFIOCITICAG	CPPCATCTGC	TEGETTECAÇE	CCICCITITI	TYSACTERCATA		CCCAATAACA	CCATATITAL
10901	TTACANOGA GO	COTATTCAA	ATAGGTOTUG	AAGGTCAAAC	ACCTAANTAT	DESTRIBUTE	CATTICARCC	TGAACCTCAA	ATACCACACAT	CTCAGTGGTA
•	•	CCATAAGTT	TATECACAGE	TICCAGITING	TESTITEMEN	COCCUATINE	GINNAGIIKAS	ACTIOGAGIT	TATECHETTA	GAGTICALICAT
19001	_	NTRANTCATO		AGTECTANAA	AAGACTACCC	CAATGAAACC	NT: TTACGGT	TCATATGCAA	AACCCACAAA	PCAAAATCO.
	OCTITIONETY 13	PATTAGTAC	GTCGACCCTC	TCAGGATTIT	TICTONIOG	CHINCHING	TACAATGCCA	AGTATACGIT	TIGGORDIT	ACTIONACT
19101	•	FICTIGIAM	GCANCANAT	GRANACKTAG	ANACTICANCT	CHIMATOTAR	TITITICION	CTACTGAGGC	AGCCGCAGGC	AATCGTCAT
	_	MGAACATIT	COTTOTTTA	CCTTTCGATC	PPTCAGTTCA	CCTTTACCTT	MANAGAGET		reactives	TACCACTAI
19201	ACTIGACTOC 19	MAGTOGTA	TIGINCAGIO		ANGATOTAGA TATAGAAACC	CCAGACACTC			ATTAMIGNAG TARTITION	GTAACTCACO
	TCAACTCAGG AT	THICACCAT	NTITICACCAT ANCAUGICAC		TICTACATET ATAITETHEG	GENCHARGAG	TATAMGAAT	CIM. COLICA	ואמווררוור	

10201	ACART-PART	CASTITABEDANT C	CTATECTICAN (CAGGCCTANT 1	TACATTRACTT T	TTAXXIN'AA T	_	_		CCCTAATATA
	TCTTGATTAC	_		-	ATTOTANCTAN A	AATECETATE A	MANATANITIA G	GATTACATAA	•	CCA1 IRING
19401	Carretterio	-	ATCCX:ACTTG	AATEXTE 1	TAGATTTOCA A	-	-	 -		ACCAT RACIO
	CCACAAGACC	_	PASCETCAAC '	TTAINGHANG 1	ATCTANAICH T	•	_	_	Ι.	
19501	ATAGANCCAG		ATCTCSTANTC	MAX.PUTTINA C	_	_	_ `		_ :	AMITTEE AAA
	TARCTIGGE	٠.	TACACCTTAG	TCCGACAACT (ה אדיאראאראיא ה	STATE TACANT C	•		TGACTICINE	
19601	TTACTOCTT	_	OTCTGATTAN	TACAGAGACT (CTTACCANAG 1	_	_		GOGANANGA	PCCTACAGAA
	ANTGACOAM	-	CACACTAATT	ATCTCTON (GAATYSTICC A	ATTTOCATT T		CTTTACCTA	CCCHINICA	ACCAROLLIA
19701	THETCAGATA	ALANTGAMI	AAGAGTTCGGA	ANTANTING (CCATCACAAAT C	_	_	CCACAAATTT	CCHOINCICC	AACATAGE(?
	AAAAGTCTAT	_	THETERACET	TTATTAMAC (GGTACCTTTA C	CTTAGATITA C	•	CCTCTTTANA	CONCATCHOO	וופועורפו
10801	POTATTICC	_	AAGTACAGTC	CTTCCAACGT	AAAATTICT (7	_	CTACATGAAC	AAGCGANTOG	TOGETECE(*)
	ACATAAACGG	_	TTCATGTCAG	GAAGGTTGCA '	TTTTAMGA (CTATEGRATIF 1	-	GATCTACTTG	TICCCTCACC	ALT GALGOLA.
10001	CENTACTICAL		ACCTTOGARG	ACCIOCICATICC	CTTINGTANTA 1	PYTACAACGT C	CAACCCATTT	MACCACCACC	GCANTGCTGG	CCIGCGCTW.
	CCATCACCTO	-	TOGARCETEG	TOCCACCAGG	GAACTGATAT A	ACCTOTTOCA O	SITCOSTANA	DOLOGICOL	COTTACCALL	GONCOLONIO
20001	CONTRACTOR	-	TOGICACTAT	GROCCCTTCC	ACATTICARTE (•	PICTITIOCCA .	TTAMAACCT	Cerrenceio	CCGGGCTCAT
	CATABURACION		ACCAGCGATA	CACCOGGAAGG	TOTANGECA (COCAGICTIC /	MCMACGGT	AATTTTGGA	CONNERGONC	×19891738
					Pstl					
			Bretta Brittani	Trabcatist	TE TEST ACAGE	TCCCTAGGNA !	ATCACCTANG	COTTOACOCA	OCCACCATEA	ACTURATA:
20102	MCALL'S ALL	Chromatala	TCC TTCC TAC	AATTGTACCA		•	TACTODATTC	CCAACTGCCT	COCTCCTAAT	TCANACTA'R:
	20100101		***************************************	COTTCACAAC	ACCEPTECA	COCTITORNOC (CATGCTTAGA	MCCACACCA	ACCACCAGIC	CTTTARCO!
20201	CATTIVACLIT		ACTION OF THE	CCARCITIC	TOXXCXCOT	_	CTACGAATCT	riccretoor	TGCTGTTCAG	DAMTTOC'1:
	GTAMCGON	•			MATTACCAA	CONFECCEANTA .	PCCATCCCT	CCCGCAACTO	OCCCCTITIC	COCCASCAGOS
20301	TATCTCTCC		CENTRACE!	TATTACECCE	TECCATOOTT		ACCTACCCCA	GOCCOTTGAC	CCCCCCAAAG	GCGCCGACTX
	ATAGAGAG		Commission			CACCETANTE	ACACCTACTC	TOCCICTATA	CCCTACCTAG	ATGGAACT'T'
20401	CCTTCACGCG	CCTTANGACT	PRECIPIOS	GTAGTGACCC	GACCCCGATT	CTCCGAATAA	TCTCCATCAG	ACCCACATAT	COGATOGATC	TACK TITCH A
			ACANGENCE	CATTACCTTE	GACTECTICITY	PCARCTICACE '	TEGCAATEAC	COCCITACTIA	CCCCCAACGA	GITTRIAMATT
70207	A PROCESSION OF THE	T CHURCHANT	TCTTCCACCO	GTAATOGAAA	CTGAGAAGAC	NOTICGACCOO A	ACCGITACTO	GCGCACCAAAT	COCCUTICET	CMMCTITAA
10206	_	•	GOSTINCIAC	GINGCCCAGE	CTAACATCAC	CMAGACTRE	TTCCTTAGTAC	ANATOCTAGE	TACTATANC	ATTORICTACK THE STANKE
70003	TICOCOM	_	CCCAATGTTG	CACGOSTCA	CATTIGTACTG	OTTTETENCE	MAGGACCATO	TITACIATICS	ALTICATALIC	CONTRACTOR OF THE PARTY OF THE
		•	ACCTACAAGG	ACCOCATGTA	CICCINCITY	AGAMCITICE	AGCCCATGAG	CCGTCAGGTG	GTGGATGATA	CINMINA
10102		•	TOGATOTICC	TYCCGTACAT	GAYAAAAAA	TCTTTCAACG	TCCCCTACTC	GOCAGICCAC	CACCTACIAI	CALL LANGE
***************************************		•	TCCTACACCA	ACACAACAAC	TCTOSATTES	TTOCCTACCT	TOCCCCACC	ATCCCCANG	CACAGGGCTA	CCC INC INC.
70007			AGGATGTOGT	JORGINSTIG	AGACCTAAAC	AACCGATGGA	ACCCCCCTTCC	TACCCCCTIC	CIGICCOGNI	
10000	THAT I SHIP	C COCTTATAGG	CANGACCTACA	CTTGACAGCA	TTACCCAGAA	AMMITTELL	TICKATCOCA	CCCTTTGGCG	CARCECAINE	ACCURITION.
* ^ 6 ^ 7	MOCCE	•	_	_	ATGREET	TTTCMMGAA	ACCETACCOT	COCHANCEOR	Grace we	

Figure ISM

21001	THATOTOCAT	THATOTOCAT GOODGCACTC	ACAGAMETERS	CASTETICASA	TCTCTACTOR C AACTECTOLT C AGAGATERTH THEREFORD		ACCCCCTACA	CATCACATTT	CTCCACCTAG	CCATCACALISA GGTACCTCCT	
11111			TOTTEGAAGE	CHETCACORG	CHCCCHCTV3.			ATCGNAACCG	TOTACCTUCG	CACGCCCTT .	
	CanadagasA	9	ACANACTICA	GAMCTOCAC	CARREACACA				AC/ "YCACGC	GTGCCCCAV :	
										ligi.	
21201	TOOCCOOCA	ACCCCACAAC	ATMAGAAGC	MCCAACATC	AACAACAGET	CCCCCATCG	GCTCCN/TIGA	GCAGGAACTG	AAAGCCATTG	TCAMGATCT	
	ACCOCCOT	F	TATTICITIES	TTCCTTGTAG	TIGITICACIA	COCHARTACC	CONSTITUTE	CONCENTION	TTTCOCTANC	ROTTICTAGA	
21301	TCOPTOTOCO	CCAPATITIT	TOCOCACCTA	THINCANGERIC	TITICCARGET	TRITTICICC	ACACAMOCTC	GCCTOCGCCA	TAGTCAATAC	OCCUPATOR.	
	ACCAMCACCC	5	ACCCCTCCAT	ACTOTTCGCG	AAAGGTTCCCA	ANCANAGAGG	TISTICATIONS	COGACOCOGT	ATCAGITATO	CCCCCCACTA	
21401	DABACTODE	COURCACTO	GATOCCCTTT	CCCTESCARCE	CHENCITIAN	MCATGCTAC	CTCTPTONGC	CCTTTOGCTT	TICTCACCAG	COACTICAACE	
	CTCTGACCCC	D	CTACCOGNAA	COGACCTTOR	CCCTCAGTIT	THETACGATG	GAGAMETEG	GCAAACCGAA	ANDACTOOTC	OCTOACTIC	
21501	AGGITTACCA	OTTTOMOTAC	GAGTCACTCC	TCCCCCCTAG	COCCATTURE	PCTTCCCC03	ACCOCTOTAL	AACOCTOGAA	ANGTECACCC	AAAGCGTAC .	
	TCCAAATGGT	ט	CTCAGTGAGG	ACCCCOCATC	GCCCTMACCA	NGANOGOGOTC	TOCCCACATA	TIGGGACCTT	TTCAGGTGGG	TTTCGCATGT	
21601	GOOCECANE	בממככמכנד	GTGCACTATT	CTCCTCCATG	TTTCTCCACG	CCTTTCCCAA	CTOCOCCCOM	ACTCCCATGG	ATCACAACCC	CACCATGAN'	
	CCCCOOOTTO	4	CACCTGATAA	GACGACGTAC	AAAGAGGTGC	CCALACOGITY	GACCGGGGTT	TCAGOGTACC	TAGACTAGGG	GTGGTACTI .	
		Y.		٠			•				
21701	CTTATTACCO	U	CTCCATCCTC	AACAGTCCCC	AGGTACACK	CACCCTGGGF	COCNICCAGO	AACABCTCTA	CAOCITICCTO	ONOCOCCAC.	
	CANTAATGGC		GAGGTACGAG	TTOTCAGGG	TCCATGRCCAG	GTCACACGCA	CCGFTCATA CC	TICHCGAGAT	GTCGAAGGAC	Chededona	
21801	COCCEACT	P CCGCAGCCAC	AGTOCCCACA	TTAGGACCCC	CACTICITIES	TOTCACTTOA	ANARCATOTA	AAAATAATOF	ACTACIACIACA	CTITCAATAA	
! } !	GCGGGATGAA		TCACGCGTCT	AATCCTCGCG	CTCAACAAA	ACACHGAACT	TITIGTACAT	TITIATTACA	TOATC ICTOT	GAAAGTTA1-r	
21901	ACCCAAATOC		ACACTETECE	GIGATIATIT	ACCECTEACTIC	THACCGRETO	COCCOTITA	ANTCANGO	OGITCHOCCO	COCATCOCTA	
	TCCOTTTACE	•	TOTORGAGOC	CACTANTAM	TRAFACTOOF	AACOCCAGAC	ACCOCANATE	THAOTHEC	CCANGACOOC	GCGTAGCGAT	
22001	STATE CALLE	THE STATE OF AGGREGACAC	OFTGCGATAC	TGGTGTTTAG	TYRCTCCACTT	AACTCAGGC	ACAACCATCC	OCCOCACCTC	COTTCAAGTTT	TCACTCCACA	
	ACCCCOTCAC	בטוכנכדופום		ACCACANATC	ACTINOCTIONA	TTTGAGTCCG	TOTTOOTAGO	COCCORCORO	CCACTICAAA	ACTGAGGGGTGT	
					EcoffV			_			
22101	COCTOCOCAC	: CATEACEANC	OCCITITACCA	CONCOCCC	CCATATCTTG	AMGTECOCAGE	TOROGECTEC	OCCC+10COCO	CGCCAOTTOC	GATACACACA	
	CCGACGCOTO		CCCMATCOT	ככשטנבבטכם	CCTATAGAAC	TTCAGCGTCA	ACCCCGGAGG	COCCACCCCC	GCGCTCAACG	CTATIOTICITY.	
22201	GTTGCAGCAC		PCAGCGCCCO	GTCCTCCACG	CHARCCAGCA	COCTUTION	CATAGATICAGA	TCCOCOTCCA	GCTCCTCCGC	GTTGCTCAGO	
	CACCICCIO	ACCTIGICAT	AGTCOCOCC	CACCACGTGC	CACCERCITE	CCCYCINACAG	CCTCTAGTCT	AGCCCCAGGT	CCAGGAGGCG	CAACGAGTCC	
22101	GCTANACAGNO	٠	TAGCTGCCTT	CCCANNAAGG	מכמנימיוניניני	ACKRETYTICAG	THECACTER	ACCOTAGTOD	CATCAAAAGG	TGACCGTCACC	
	CUCHICCETC	<		COGNITITIES	ניםכניכעכעטשט	TCCGAMCTC	AACGTGAGCG	TOCCATICACE	OTAGITTICC	ACTRACCACIO	
22401	CONTRACTOR OF THE PARTY OF THE	U		TAMARICETT	GATCTOCTTA	AMARICCACCT	GASCETTISE	GCCTTCAGAG	AAGAACATGC	COLUNGACIT	
	GCCAGACCCO		_	ATTITICOGAN	CTAGACGAAT	TITCGGTGGA	CTCCGAAACO	COGNAKA:TIC	TICTIGING	THETERA	
)	{				lighti.				
22501	OCCOGNAAC	-	GATTCCCCG GACAGGCCGC				מפתמאדו זית	ACCACATITE	GGCCCCACCG	CANCANCACA	
	COCCETITION	3 ACTINACEDEC	CTGTCCGGCG	CAGCACOTGC	GTCGTAGAAC	GTACCACA	CCTCTAGACG	TGGTGTANAG			

Figur 15N

•		Section 1	Control of the Contro	GUSTALIST		CYTH ACATEC A	ATTICAATCA	CONOCICCITY !	ATTITATION !	ATGCTTCCGT
10977	ANCTIMENT		CHANGE TO SERVICE						TAMATAGINI	TACGNAGGICA
	TAGAACCIAGA	ACGAILIGH	3							?
***			and part and a second	THE PROPERTY OF THE PARTY OF TH	rwarehrahe .	מימיבאמיכרים	TOCOCTUCTO	ATCCTTCTAG	GICACCTOTO	CNANCANTO
72/01	GTAGACACTT	AMACTICACT	POLICE ICAG						CACTCCACAC	GTTW:CTK:W"
		Pet								
22801	Thomas are	TYST ACTORAGE	(2) CATCAT	COTCACAAAG	CICTION TICE	PKXTPKTANCKTF (CAGCITICAAC	ccoccoract	CCTCGTTCAG	CCM3OTCT1.1
	GTCCATGGGG	ACCITICATING	CGCCGTAGTA			ACCACTTORA (GICCOACCITIO	GOCGCCACGA	GCACCAAGTC (GENECACIANC
12901	ratarday	CAGAGETTA	CACTICACTOR	CCAGTAGT	TICAMETERIZE	CTTTAGATEG .	TIMECACOT	GGTACTTGTC	CATCACCGCG	CINCINCAGENT
10671	GTATGCCGGC	GETCTCGAAG	GTGAACCAOT			GNAATCTAGE	AATAGGTGCA	CCATGAACAG	GTAGTCGCGC	acaconcor 1
			2	•						
23001	CCATCCCT	CHCCCACOCA	GACACCATCG	GCACACTCAG		-	CACHITCCGC	THE CALTOONIC		CCICTICAT
1	OUTACOCODAA	GAGGGTGCGT	CTGTGCTAGC	CONCINCACTO	CCCCAAGTAG	TOCCATTANA	CTCAAACCCC	NAGCCINCCCG		COMONACIO
23101	CCCCATACCA	COCOCCACTO	OCITICALE		PECACTOTAC	CCTTACCTCC	TTTCCCATCC	TTCATTAGCA	_	GCTRIANACTO:
1	GCCSTATOST	GCGCGGTGAC	CCAGCAGAAG	TAMGTECOCE	CCCTGACACG	CONNTINGALID	MACGCTACG	AACTAATCGT		CONCIENCE
23201	ACCATTIGIA	GCCCACATC	TICICITICS	TCCTCCTCT	CCACCATTAC		GACCIOCICCE	COGOCINGO	ACAAGGGCGC	TICTITUE
2	TCCTAAACAT	COCCONTATAG	ARGAGAAAGA	-	GITTOCTANTO	GAGACCACTA	CCGCCCGCGA	ACCCCGANCCC	TCTTCCCOCG	ANGANANGA
10556	TETTINGSCOC		Tecaceacca	ACCTOCATOD	CCACCACACTC	CONTRICTOR	GCACCAGCGC	GICTIOIGAT	GAGICTICCE	CONCCINCODA
	ACAACCCGCG	-	AGGCGGCGCC	TCCAGCTACC	OGCIGCCCGAC	CCACACCCCC	concenced	CRGAACACTA	CTCAGAAAGA	OCAGOTA OCCT
LUYEC	THE STATE OF	CATCHER	OCT TOTAL	0000000000	CONTROCTO	CCCACCOCGA	CHARGACTAC	ACOTECTICCA	1001100000	Acorodom.
40404	CAGTATAGG		CGANAAACC	_	CCTCCCCCCC	מוכוומנונות	OCCULTOCTO	TOCAGGAGGT	ACCAACCCCC	POCAGCOC
11541	Car and Carlot	Contaction .	CONCOUNTED	_	CTTCCCCACT	GECATTICC	TICHES TATA	GOCAGAAAAA	GATCATOCHO	TCAGTCGAL:A
40552	CONTROCOCAG		CCACCANGC		GANCOCCTCA	CCOCTAAAGG	ANGAGGATAT	ссотстти	CTAGTACCTC	AGTCAGCTCF
12501	POPEGGE PE		recreating of		Chretecace	GATGCCGCCA	ACCCCCCTAC	CACCITICCCC	GPCGAGGCAC	CCCCCCTTGA
400C7			GGGAGACTCA		GCIACACASTIVAS	CTACGGCGGT	TRICECCIONTO	OTOGAAGGGG	CAGCTCCCTG	COCCCCAACT
10110		CENTER PERSONAL	ACCRECACT		ACCGMENCO	ACCAGGACCO	CTCAGTACCA	ACAGAGGATA	AAAAGCANGA	CCAGGACAN'
10.63	CCICCICCT	CACTAATAGE	TEGITECTICOS		TCGCFFCTGC	TCTCCTVGC	GAGTCATOOF	TOTOTOCTAT	TITIOCITICS	COTCCTOTTO
11001	GENERALIZATION	ACTIVITIES	AGTCGCCCC	GOOGACCAAA	COCATOCCA	CTACCTAGAT	CTOGGAGACO	ACGRECATION	GAAGCATCTG	CAGCGCCAGT
10867		TREFERENCE	PCAGCCCOCC		CCCTACCGCT	GATOGATCTA	CACCCTCTOC	TECACCACAA	CTTCGTAGAC	Gregodorea
,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,			-	_	accectoace	ATAGGGGANTO	TCAGCCTTGC	CTACGAACGC	CACCTATTICT	CACHOCOCCT
70667	CECENTRAL	GACOCTOCOC	-		CTANCAGGGG	TATCGCCTAC	NGTCGNANCO	GATGCTTGCG	GYCCATAACA	OTCOCOCOCA
•		446044	ACCOUNTS	CCACCCCAAC	CLCCCCCCCACA	ACTICTACC	CUTATTRACC	GTOCCAGAGG	TOCHROCCAC	CTATCACATE
10047	THE STATE OF THE	COCCUMENT.	TOCCUTOTAC	_	COCCCCCACT	TCAACATCITO	CCATANACOO	CACGOTOTOC	ACGAACGOTO	GATACTTA:
10170	September 1888	A ACTION AAGAT	ACCCTATCC		TRACEGIFFECEN ACCECANGETON ARCEGIACANG	ARCGRACANG			CGCTGTCATA	CCTCIATATO:
77769	AAAAAGGT				TGGCGTCGGC	resectoric	GTCCACTGGA	ACOCCOTOCO	CCGACAGTAT	GGACTATAR

Figure 150

pmiradsgag mer682

14201	W. Market St. B.	COARCTCA	AAAATCTTT	Acceptant	Architecturing	AMericane	CAAACCETTE	CCANCACACA	AACAGCCAAA	ATGAMETCA
1034		-	200000000000000000000000000000000000000		- Table 12 - Albert 12 - Alber			Contraction	Preprietar	TACTUTE
			The state of the s	יירר אנשמייר	ייייייי אלי וייייי			, , , , , , , , , , , , , , , , , , , ,		
		ŧ	AINO							
24301	CTCTCCACTO	TTGGTGGAAC	TOTARGETER	CAACIRCATIST	CTAMICCITAC	TAMANTECAG	CATCEAGGEC	ACCCACTITO	CCTACCCOCC	ACTTAACCTA
	GAGACCTICAC	ARCCACCTTO	AGCTCCCACT	GETTERSTRUCTED	GATUBGEATG	ATTITION	GTARCTCCAG	TYXXTCAAAC	GCATGGGCCG	TRANTIGGAT
24401	CCCCCCAAGG	TCATGAGCAC	AGTCATGAGT	GARCTICATOG	THEOCHAC	מנאטרטבינו	CACACOCIATO	CAATTTGCA	AGAACAACA	כענכעעסטי
	GOOGGATICE	AGTACTEGTO	TCAGTACTCA	CTCCACTARC	ACCATORCACG	CHARTERING	CHCTCCCTAC	GTTTAAACGT	TCTIGITIO	CHUCHCCCINI
24501	TACCCCCAGT	TOCCOACGAG	CAGCTAGICG	CENTRACTICA	AACGCGCGAG	CCTURECTACT	TREGARGAGED	ACCCAAACTA	AFGATGGCCG	CAGT :CTCT:T
	ATCCCCTCA	ACCOCHOCIC	GTCGATCGCG	CCACCCAAGT	THICHDOCHC	CONCORCITOR	ACCTCCTCGC	TOCOTTTGAL	TACTACCOGC	GTCACGAGCA
		5	4							•
24601	PACCETOGAG	CTTCAGTGCA	TGCAGCGGTT	CTTTGCTTMC	CCCACACATEC	ACKCELAACKT	AGNOTANACA	TTCCACTACA	CCTTTCGACA	COCCTACGTA
	ATGCCACCTC	CHACTCACOF	ACOTOCCCAA	CANACCACTG	GGCCTCTACG	TCGCGTTCGA	Tercentur	AACOTOATCT	GCAMACTICE	CCCGATCCAT
		m A B								
24701	COCCAGOCCE	8	CAACGTGGAG	CTCTGCANGC	TRESTUTE	CCPTAGGAATT	TTGCACGANA	ACCOCCTTOO	GCAMACGTO	CFTCAFFCCA
	GOODICCOOL		GTICCACCITC	GACACOTTOG	ACCAGAGGAT	GCAACCTTAA	ACCIOCITY	TOCCOGNACC	COTTITICCAC	GAAGTAACOT
		Asci	ì							
24801	COCTCAAOOG	CGAGGCCCCC	COCCACTACO	TCCCCCACTO	CGTTTACTTA	TTICTATICET	ACACCTIGGCA	GACOGCCATO	OCCUPACIO	AGCAGTGCTT
	BCGAGTTCCC	OCTOCOCOCO	OCCURATOR	AGCCGCTGAC	GCANATGAAT	AAAGATACGA	TUTOGACCGT	CTOCCOOTIAC	CCOCMACCO	TCGTCACGAA
			Pan							•
24901	OCAGGAGTGC	MICHENAGO	AGCTCCAGAA	ACTCACTARAG	CANANCTION	ACTACCTATO	CACCICCTTC	ACGAGCGCF	CCCHIGGCCOC	OCACCTGGC':
	CCTCCTCACO	TICCAOTICC	TCGACGITCTT	TGACGATTTC	GTTTTCAACT	TOCTOGRAFAC	CTOCCOOMB	TIGCTCGCGA	GGCACCCGCC	COTOGACCOC:
25001	GACATCATTT	TCCCCOMCG	CCTGCTTAAA	ACCCTUXCAAC	ACKASTICTURE	ARACTTCACC	ACTCAAAGCA	TOTTOCAGAA	CTTTAGGAAC	PHENTICETA"
	CTOTAGTAAA	AGGGGCTTGC	GGACCEANTET	TOCCACCITIC	TCCCAGACGO	TCTCAACITOG	TCAGTTTCGT	ACAACGICIT	GANATOCITIG	AAATARKATK.
25101	ACCOCICAGO	ANTETTOCCC	GCCACCTOCT	GTGCACTTCC	TACCOACTIF	CHICCCAPTA	ACTACCCCCA	ATGCCCTCCO	CCGCTTTGGG	GCCACACTA
	TEGEGRAFICE	TTAGAACGGG	COSTOCACGA	CACGTGAAGG	ATCCCTGAAA	CACGGGTANT	TCATGGCGCT	TACOGGAGGC	OCCCARACCC	COCHCACCAT
	- Dec	2								
25201	CCFFCTGCAG	CTAGCCAACT	ACCPTOCCTA	CCACTICTICAC	ATAATCCAAC	ACCTGAGCGG	TCACCOTCTA	CHORONOTOTIC	ACTOTOCOCTO	CARCTATKIC
•	CONCACCTO	GATCGGTTGA	TOGANCOCAT	GCTCAGACTO	TATTACCTTC	TOCACTORCE	ACTOCCAGAT	GACCTICACAG	TGACAGCGAC	GTTCCATACY:
						2		Pul		
25301	ACCOMING	OCTCC:1007	TICCAATICO	CACCTCCTTA	ACCARAGICA	ANTTATCGG	ACCTITICAGE	TOCAGGGTCC	CTCCCCTGAC	GAMMETICET
			AACGTTAAGC	GTCGACGAAT	TOCTITICAGE	TTAATAGCCA	TOGARACTEG	ACOFCCCAGO	GAGCGGACTG	CTTTTC/GG/
25401	CERTIFICATION	STERANCIC	ACTECCADOOC	TOTOGACGTC	CACTTACCTT	COCAMPTED	TACCTGAGGA	CTACCACCCC	CACCAGATTA	GOTTCTACCA
	GCCCMOCCC		TCACCCCCC	ACACCTGCAG	CCGNATGONA	CHICATTERNAC	ATCASACTOCT	CATCCTOCGG	GTCCTCTAAT	CCARGATIXT
25501	AGACCAATCC	COCCCOCCTA	ATOCOGNOCT	TACCTACCTURC	CHIATTACCC	MASSICACAT	TCTTRACCAA	TICCINGCCA	TCAACAAACE	CCCCCANON
 		_	TACCCCTCGA	ATGCCCCCATT	CANTAATYCG	PCCCONTGEA	ACANCCOCTT	AACCITICOGT	AGTIGITACO	הממנידוכיוו
25601	TTTCTCTAC	GANAGORACO	GOODTITAC	THYCACTOCC	AUTRICICIONICA	OCACCTCANC	CCAATCCCCC	COCCOCCOCA	GCCCTATCAG	CANCARRITA.
1 1	AAACACGATG	CHICCORD	CCCCCAAATO	AACCTCACAGG	TCACCCCCCT	CCTCGAGTIG	CCTTACKXXCG	ACCIONACET	CCAXCATAGIC	GICCICOCO

Figure 15P

phrkactsgag Nergb2

25701	3000001100	Trecevent	CCCACCCAAA	AAGAAGCTCC	NETRENTER	נציראנדראת האבמאמחאמ	האכמאמהאמ	AATACTOCICA	CACTCAGGCA	CACCACACTET
٠	CCCGGGAACG	AAGGGTCCTA	CCORGANITIE	TICTICGACG		CINTRACTURE	CINCICCITEC	TTATCACCCT	OTCACTCCGT	CTCCTCCN.
						1 tov (II)				
25801	TOCACGAGGA	GCAGGAGGAC	ATCATCANG	ACTICACIANA	נינידאמאניינאק	מאאחנידדכבה	AGGTCGAAGA	actorcadac	GALACACCOT	CACCTTON .
	ACCIDENCE	cerecteero	TACTACCTTC	TGACCCTRITE	CONTRACTOR	CTTCGAAGOC	TCCARCTICT	CCACAGTCTG	CTTTGTGGCA	פונספינאפטייא
25901	CGCATTCCCC	Tesecoscoc	CCCARANATC	GCCAACTGGF	TECARRENTER	CTACAACCTC	CHICTCHTCAG	OCCCCCCCCCC	CACTGCCCGF	TCIRCCACCC
	OCOTINACIOCO	AGCGGCCGCG	CONTENTING	CCONTRACCCA	ACCITICATACC	GATGITISCAG	CCGAGGAGTC	crececeseé	OTCACGOGCA	ACCCCTCC
26001	AACCGTAGAT	GODACACCAC	TOGANCCAOR	OCCUGINAGE	CCNACAGEC	GCCGCCGTTA	CCCCAAGACC	NACANCAGEG	CCAAGOCTAC	CIXCICATOO:
	TTOOCAICTA	CCCTGTGGTR	ACCTRIGGREE	CGGCCATTCA	GGTTCOTCGG	CHRICHACANT	COGGINCICS	TICTICICOC	GGTTCCGATG	GCGACTACCO;
26101	OCCOOCACAA	GAACGCCATA	-	TOCAROACTO	TEXORGENICAAL	ATCTOCTION	CCCGCCGCTT	TCTTCTAC	CATCACORCO	TUXXI THEFT
	COCCOTOTT	CTTCCCCTAT	CHACGAACGA	ACCITICTION	ACCCCCGTTG	TAGACICANC	GCCCCCCCAA	ACANGAGATO	GTAGTGCCGC	ACCURANCE!
26201	CCOTARCATC	CTCCATTACT		CTACAGCCCA	TACTRICACCI	GCCGCCACAGCGG	CAGCNACAGC	ACCOCCACA	CAGAAGCAAA	GREGIACEOGA
	OCCATIGITAD	GACOTANTOA	TOOCAGTAGA	CATOTOCOCCT	ATGACGTGCC	COCCETCOCC	GECOFIGICA	TCGCCCOOTGT	GICTICOTITE	CCGCTGGCCT
26301	TAGCAAGACT	CTGACAAAGC		CACAGCGGCG	GCARCARCAG	CACCIANTIACC	ectecencia	GCGCCCAACO	AACCCOTATC	CACCCCCCAAA
	ANCOTHCTOA	CACTOTITICO	GGTTCTTTAG	GIGICOCCOC	conconcon	CTCCTCCTCG	CGACCCAGAC	COCOCOTIOC	TTOOCCATAG	CTOGGCGCTC
26401	CTTABANACA	GCATTITICS	CACTCTATAT	CCTATATTEC	AACAGAGCAG	GOCCANOA	CANGACCTUA	ANTAMAM	CACCITCITO	CCATCCCTCA
	GAATCTFIGT	CCTANANGO	GTGAGACATA	CCATATAAAG	THENEROPIE	CCCCGGTTCTT	GENCINCARCE	TITATITI	GTCCAGAGAC	OCTACOGAGE
26501	CCCGCAGCTO	CCTOTATCAC	ANAGCGAAG	ATCARCTTCG	GCGCACGCTG	האחחההכפס	AGCICICITY	CAGTAAATAC	TOCOCOCTOA	CTCTTAAGGA
	GCGCCTCGAC	CCACATACTO	francocrac	TAGTCCAAGC	CCCCTACCTAC	CTTCTGCGCC	TCCGAGAGAA	OTCATITIAND	ACCCCCCACT	GAGAATHCC .
26601	CTAUTTICOC	OCCUPACIO	AAATTTAACC	CCCONNCTA	CGTCATCTCC	AGRICACCACA	CCCGGGGGCA	OCACCTOTTO	TCAGCGCCAT	TATHARCAM
	GATCAAAGCG	CODGANAGAG	TTTARATTOG	CGCTTTTCAT	CCACTAGAGE	TCCCCCCCTCT	OGGCCGCGGT	CCTGGACAAC	AGREGEGGTA	ATACTOGITIC
26701	GUANTICCCA	COCCCTACAT			TGGGACTICAC	COCTGOAGET	CCCCAACACT	ACTCAACCCG	ANTANACTAC	ATGARCCCC
	CTTTAAGGGT	GCGGGATGTA	CACCTCAATG	Greenent	ACCCTGAACG	CCCACCTCGA	COCOTTCTGA	TCAGTTGGGC	Tratttcato	TACTCGCGCC
		FOOT			ű (Econi				
26801	GACCCCACAT	GATATCCCGG				CCCAAACCCA ATTCTCCTCG	MCAGACACC	TATTACCACC	ACACCTCOTA	AFAACCTTAA
	CTOSOCIOTA	CTATACACL		ATECECASE	INVESTIGATION OF THE PROPERTY	ואיטאיטיירו	וניונינינים	3		TAN
26901	TCCCCGTAGE	TOCCCCACTO	cccroatera	CCAGGAAAOT	CCCCCLCCCA	CCACTCTOGT	ACTTCC(:NGA	GACGCCCAGG	CCONCTICA	CATCACTANC
	AGGGGCATCA	ACCERECEAC	GGGACCACAT	OGTECTTICA	GOCCANORIT	OTTRACACOL	TOMOGRACT	CIGCOGGICC	GOCTTCANGT	CFACTGATTG
27001	TCAGGGGGGC	AGCTTGCGGG	COCCUTICGE	CACAGOGINE	ממוכנוכנונו	CCACACATATA	ACTCACCTGA	CANTCAGAGG	OCCADOTATE	CARCTCAACT
	Agreeceded	TEGANCOCCC	GCCGMAGCA	OFFICECCACG	מכאונכונומנוכנ	COTCCCATAT	TOAGNOONCT	GITAGICICC	CCCTCCATA	GPCCAGTTAN.
27101	ACCINOTOCOCT	GAGCTCCTCO	CHIOCIETEC	GICCGGACCEG	GACATTTCAG	ATCGGCGGC	CCOROCCIACTO	THEATTENED	CCTCGTCAGG	CANTCCTAAC
	TOCTCACCCA	CTCGAGGAGC	GANCCACAGG	CAGOCCTGCC	CTCTAAAGTC	TAGCCGCCGC	OCCUBECTORS	AAGTAAGTOC	GCACCACTCC	GTTAGGATTG
	Pyti									
27201	TCTGCAGACC	restectes		TOGAGGCATT			GEAGTITICHS		ACTITAACCC	CHICHCOOLA
	AGACGACTOG	AGCAGGAGAC	TCOCCCCCAG	ACCTCCOTAA	כנידוהאהאהה	TTANTANCT	CCTCANACAC	CCTACCCAGA	TCAAATTOCO	GANGAGGCCT

· Figure 1562

GCAGAGCAAC	CONCINCULTY	ATATCTAGES		CICINCACIOCO: IA	ccrorce		AGNATTA:A	TCTTTAAT	Afterence .	THENCHOOS:	TCCTTACCT:	ACCIVATOGAC	TOTTTACCAG	ACMATOSTC	CTATTICTAT	CATAACAT		OCCIOCITY :	COCACCACAC	GECYCLECYC	CACTCCCCFT		ACCACAGN .	reoreren's	TTACAGTT1'F	AATGTCAAAA	•	GHOSCCCCCA	CACCGRORGE	TACANAMICA	Atcressor .	ACCANTTY ?	FICTFIAN:T	ATATIXCTC1'A	TATACCAC ;T
AAGTOCAGAO	TICACCTOTIC	CCCCACACATC	2000	TACTTOROCO	ATCAACTCGC		TAATAAATAC	ATTATTATO	TACTITITAC	ATCANAATTO	AACACCACCC	Tronochago	TCANTAACTC	ACTTATICAG	ACTICTACOOD	TOAGATOCCC		MODETER	Treceages	TCACCCTTGC	ACTOCOLACO		TATAAAATGC	ATAILLIAGO	CACTATAATO	CTCATATTAC		AGTATAAGTT	TCATATTCA	CTATATTAAA	CATATAATIT	CFOCFFICCAA	DACCAACCIT	ECTATIGIOGO	AGATACACCC
ACTOANTOTT	TRACTTACAA	CTITICANTTO	SACE DANS	כפככככבומכ	GCCGGGGGACG		GROCHEROTA	CACGACTCAT	CCTINCCTOG	CCAATCCACC	CATCAGAAAA	GPAGICTITY	COCACAGACC	GCCTGTCT00	AATTCAAOCA	THAGTICOT		TICHCROCCE	ANCAGACCOA	CTAGGTTTAC	GATCCAAATG		GCACCACTCT	CCTCGTGAGA	TCACACTACA	ACTOTOATOT		ATCAGCAMC	TACTICOTITIO	GTACCCTACT	CATGOGATGA	OCHUPACTOO	CGANATOROC	MCMITTON	TIGITAMCTO
GACGGCTACG	これにいているものに	ACTITION	TCAMPALICAT	GTTTACCCAG	CAAATGGGTC		TOCCATCICT	ACCCTAGAGA	CCANGGGAA	CONCOCH	TCAGCTACTC	AGTCGATCAO	AGACTTTTC	TCTGAAAAA	GITTATGAAC	CAAATACTTO	٠	ATACTAACCC	TATGATTGCG	GTACATAATC	CATCTATTAG		GCTANTGAGT	CCATTACTCA	THE CHOCK HOLD	ccorcoance		TACCATGTAC	ATGGTACATG	CONTRACTOR	CCANACCAGA	ACCACTANCT	TEXTENTED	CATTCCCCTO	GTAAGGGGGAC
CCACTCCCC	ניבוניאטכניזכ	CACTICAGE	CTGACACCAC	TCATTCGGGA	ACTAAGCCCT	Chatti	AGATCTTTGT	TCTAGAAACA	CCCAAGCAAA	GOOTTCOILT	CHCTCCCAOC	GAGAGGCTCG	ACCUTABACC	TESCATTING	CTACTGICKIO	CATCACACCC		CHITATRETT	GANNTARGAA	NGATGATTAG	TCTACTAATC		COCAGCTGAA	GCOTTCGACTT	TATGCTATT	ATACCATAAA		TOTOGOGOLAT	ACACCCTGTA	TACAGTGCTC	ATCTCACGAG	ACCTANTETE	TCGATTACAG	TRETERATAC	ACGAGITTATG
ACCITIZETAM	TOCKET:AFFF	CTTMALCRIA.	מאאל נאאנים	מכככנים אנאיכ	CCCCCATCGG		GATTACATCA	CIMITATIAGE	Trittinedeces	MINNSTORGE	ACCIACIACIANC	TGCTCTCTTG	CTACCOCCTG	GATGGCGGAC	NAVICERCAG	TTACCGCOTC		TRATORETET	AACACTANGA	TCGCCACCCA	AGCGGTGGGT		ATCTTACATT	TACAATGTAA	GEARCETOTT	CATACCACAA		TITIATICAAA	MANTACTIT	CTATGCTAAT	CATACCATTA	TANCTTACAA	ATTCAATCIT	CCICATATIC	CCAGTANAGG
CCTAACTTAG	CCATTGAAAC		COCHCATICAC	COGAGACTT	CCCTCTCGAA		CCTAACCCTO	GCATTGCGAC	AACTCCACCG	TTCCGGTGGC	GACTICACTET	CTCACTCAGA	TECACCACAC	Acordonard	OTATTAGGCC	CATAATCCGG		AFTCTCTGTC	TAAGAGACNG	AACGCTGGGG	TTCCCACCCC		CCAGCCTGTA	GOTCGGACAT	AAATTOCCAA	TTTMCCGTT	£0.	TACTITITICEA	ATGAMANGOT	TOCTOCACTO	ACGACGTGAC	CTTAATTTAC	CAATTAAATO	TAMCCCCCC	ATTTOCOCO
TCAATTTAT	AGTTANATA	-	GTGACAGCGG	TACCGCCCA	AATGGCCCCCT		TTGCAACTGT	AACCITICACA	CCATCCTGTA	GGTAGGACAT	AACCCAGACG	PROGRETICE	CACCOCCOC	GTGGCCGGCG	MCCCTTAGG	TTGGGAATCC		COPTICACETY	ECAACECEA	CACCITITION	GTCGAAAAT		TITTANGGAG	AAAATTCCTC	CACAAAAACA	GIGHTHOP	Religh	CHITTATOTA	GAMATACAT	TOCCACTETIC	ACCOTCAARG	AAGAAAATGC	TICTITIACG	CABTACOATT	CTIVICCIA
ACTATCCOGA '	TGATAGGCCT	-	TOTOGACCAG	OOCONCCOCC .	CCOCAGGCCG		TCACTGIGAT	ACTOACACTA	CETECTATES	CCAGCATAGC	CAACAGITIC	OTTOTORAND	ACCIACIOCOT	TOCTCACGCA	GNOCTTAGAA	CTCGAATCTT	Xtbs1	CTROMATOGO	CATCITACC	CATTTATTOT	GINANTANCA		AAAAGGTGGA	TITTECACCE	GETTATICAL	CUANTARGED		ACTICATABA	TCAGTATTT	TETAAAACAC	ACCEPTION	TATTICACIAA	ATAACTOTT	ATTACABLE	TARTATAT
CCHCCCOOCC	8	_	ACCOCCCACTT	CCCOGCGCAC	COCCCCCTO		ccetoranc	GOODCACANG	ATATACTOOD	TATATOACCC	CTOTOATTTA	GACACTANAT	CCGGGAACOF	GCCCTTOCA	AACAGGAGGT	THGTCCTCCA		TCAGGTTTCT	AGTCCANAGA		ACCTOTANC	Kprs	CATTAINTAINT	CCATGGTGGG	ATTERABARET	PACTITICGA		CCAGGGGTAAA		CAAAATHETG	GETTENCIC	CAN'S ARTH		SAS MITTER BUT	AMAGEST TARGE
27301	1	27401		Z7501			27601		27701		27801))	27901	1	28001	 		28101		28201			10186		28401	•		28501		28601		78701		10000	70997

. 28901	COCCATOTIC	CTTGAAGTCA	CCTMCCATC	ATCACTCACAT	CACTEMANCE	CCACACACCTO 1	TCCCGCCGAT AGGCGCCTA	THUTTICCAGT ANCANGETCA	CCAACTACAG	CCACCCACTC CCT 125T 111
29001	TAACAGAGAT	GACCAACACA CTGGTTGTGT	ACCAACGCGG	בבפרבמרדאר מכניסבמאזוי	CCCACTTACA	ACATIVEDON I	ATACACCCCA TATGTGGGGT	ACTITICTUCE	TTTOTCASTA AACAGTTAT	ACTIGORATAA TOACCCTATT
29101	CTTOOCCATO	TOGTOGTTCT ACCACCAAGA	CCATAIXGCT	TATISTA	TRACTITATION	TTATCHEATT ANTACACTUR	CATCTRICTEC (STAGACG)	CTAAACCCCA	AACOCIECEO	ACCACCCATC TOGT KYTTAG
29201	TATROTCCCA	TCATTOTGCT	ACACCCAAAC	AATCATCAAA	TOTATAGATT			Territerer	TACACTATCA	TTAATCACA
	ATATCAGGGT	MGTMACALCUA	orresonor.	TINCIMETIT	ACASTATCTAN	ברומירוה	A PARTOLINE	A CHARLANGAIC A	7	171111111111111111111111111111111111111
29301	CATCATTCCT	CCACTETITA	TATTACTORC	CCTICATICCE					TCOMCTAGA	CTGCATTO A
	. STRCTANGGA	GCTCAAAAT	ATAATGACTG	CCANCANCOC	CAANNANCAC	CCACCARRETG	TANCCGACGC	CAAAGAGTGT	AGCINCATO	GACOTANO .T.
					8.4 2	- F				
29401	accincacao				CACTEMIENT CACCITEMIC	CACCCACATC	ACTOTOGRAM ACCOUNTAL		CCAGNGCATT	GACTOGGTA:#
	CCCAMOTOTC	MCA TAUMACKA	MINCING		ברניותי ואייוטר		Leaf			
29501	GIGTOCOCYT	TOCATATOTO	AGACACCATC	CCCAGINCAG	GRACAGRACT	ATACCTGAGC	TICTIAGNAT TOTTTANTER		TOWATTING	TOTOACTTY
	CACACOCCAA	ACCTATAGAG	-	_	CCTGTCCTGA	TATEGACTER	ANGANTETTA	ACALATTAAT	ACTITIVAMIO	ACACTOMAN
29601	CTCCTCATTA	THICKACCT	ATCTGCGTTT	TOTTCCCCGA	CCTCCAAGCC	TCAAAGACAT	ATATCATGCA	CATTCACTCG	TATATCESART	ATTCCAAGIT
	GACCACTAAT	AAACOTOOGA	TAGACCCANA	ACANOGOGGT	GGAGGTTCGG	AGTITICTGTA .		CTAMOTONOC	ATATACCTIA	TAACCITICAA
	•		•				Pal			
29701	OCTACANTER	AAAAAGCGAT			TOCARTCATC		TOTTICTIOCNO	TACCATCTTA	OCCUPACEA COCCUPACEA	TATATECEIA
	CONTOTTACT	TTTTCCCTA	GAMAGGETTIC	CONCCANTAT	ACCTTAGTAG	AGACAATACC		ATCHARGET	COCONICORI	HIMMAN
29801	CCTTOACATT	OCCTOGNACE	CAATACATCC	CATGAACCAC	CCAACTITICC	כבפכשכבנפב		CTGCAACAAG		COCTTICH
	COARCIGINA	CCGACCTIGC	GTTATCTACG	GIACTIGGIG	COTTONARO	٥٥٥٥٥٥٥٥٥	ATACGAAGGT	GACGITIOTIC	AACAACGGCC	GCCCBAAry 1
		•							Both	
19901	C. B. C. B. M.	Accrete	ACCTICIOCC	ACCCCCACTO	AAATCAGCTA	CTTTAATCTA	ACAGGAGAGA ATGACTGACA		CCCTADATCT	AGAAATCCAC
		TOGRACOCO			TTTAGTEGAT	CANATTACAT	resecteent	TACTOACTOF	GOGATETAGA	retitraceto
10001	OCAATTATTA	CNGAGCAGCO	CCTGCTAGAA	AGACGCAGG	CAGCOOCCCA		ATGNATCAAG	AGCTCCAAGA	CATOOTTAAC	TTGCACCAGT
	CCTTANTANT	grenconce	GCACGATCTT	1CTGCGTCCC	GTOXCOACT	correspond	TACTTAGETIC	respectives	GTACCAATTG	AACOTOTO
30101	GCMANOGO	PATCHETOT	CTCGTAAACC	ACICCAMOT	CACCTACGAC	-		CCTTAGCTAC	AAGITICCCAA	CCANGCOT"
,	CENTITICCCC	ATAGAMAACA	GNGCATTICO	**************************************	CTOCATOCTO	-		GENTOCATO	TECANCOURT	GGT FILTO AL.T
30201	DANATION	GPCATGOTOG		_			CGAAGGCTGC	AFFCACTCAC	CHTCTCAACC	ACCTCARGAT
	CITTAACCAC	CAOTACCACC	cicrimos	Bull	Market Color	W.C.W.C.I.I.O.				
30301	CTCTOCACCC	TTATTMAGAC	CCTGTGCGGT		TATICCCT			THANGCATCA	CITACITAAA	NTCAGTTAGC
	cadacortoco	AATAATTCTG	GACACGCCA		GAGTITICTAG AATAAGGEAA	ATTCATTATT	TITTITI	ATTICGINGT	GANTGANTIT	14.104.10

PMRKAdSqag MERGBZ

10401	AAATTIK TITT	CCAGHITATE	CARTAGEACT			CHASTATAGE	AGCTTACTOC	Tracendena	Carried Action	ARTCTABATE
1	TITANAGACA	_	-		GUNGARTEGA	CACT: ATABCG	TYCANGRAGG	ACCOACOTET	CAAACACOTO	TTAGATTTAC
30501	CANTIGECAGE	TECTOCHET	TCCTGTCCAT	CCOCACCCAC	TATCTFCATG	THEFT	TGAAGCGCGC	AACACCOTOF	GAACATACCT	TCAACCCC:T
	CTTACACTICA	AACCACCACA	ALXGAL! AGGTA	CAXICTIONS	ATAGAAGIAC	NACAACITICT	ACTITICAGGG	TTCTGGCAGA	CFTCTATOGA	ACTITICISES A
30601	GTATCCATAT	GACACOGAAA	CCGOTCCTCC	AACTIGTICCT	THETTACTE	CICCCITIGE	ATCCCCAAT	OCCUPACAGO	AGAOTECECE	TOCCOTACT :
	CATAGOTATA	Chorocont	OCCCAGGAGG	THEACACGGA	ANGANTONG	GACCCANACA	TAGGGGGTTA	CCCAAAGTTC	TCTCAGGGG	ACCCCATGAG
				J.	Sict					
30701	retricedec	TATECCAACC	TCTASTTACC	TCCANTISCA	TKICTTOCOCT	CAAAATCOGC	AACTGRETET	CTCTGGACGA	GCCCGCCAAC	CTTACCTCCY:
	AGAMCOCOO	NTAGGCTTGG	ACATCANTEG	ACCITACCGI	ACGNACOCGA	GTTTTACCCG	TTOCCOGRADA	GAGACCTGCT	CCGGCCGTTG	GANTRICAGE 3
30801	AAAATOTAAC	CACTOTORGE	CCACCTCTCA	AAANAMCCAA	GTTANKTATA	AACCTOGAAA	TATCTYR.ACC	CCTCACAGET	ACCTCAGAAG	CCCTANCTICT
	TTTTACATTG	GYCACACTCO	COTOCACACT	TITITUGGE	CASTITICIAT	TTCGACCTTT	ATAGACG1033	OGAGITGICAA	TOGAGICTIC	GOUNTIDACA
30901	000700000	: GCACCTCTAA	TOGICOCOOD	CAACACACTC	ACCATTACAAT	CACAROCCCC	CCTAACCGTG	CACGACTCCA	AACTTAGGAT	TOCCACCCAA
	CCGACGGCGG	COTOGAGATT	ACCAGOGOCC	GENERATIONS	TOSTACOTTA	GTGTCCGGG	CCATTICCCAC	GTGCTGAGGT	TTGAATCGTA	ACOGNOCOT I'
31001	OGACCCCTCA	_		GCCCTGCAAA	CATCAGGCCC	CCTCACCACC	ACCGATAGCA	GTACCCTTAC	TATCACTOCC	TCACCCCCTT
	CCTOOCOMOT	P GICACAGICT	TCCTTTCGAT	CGGGACGTT	GTACTCCCCC	OCACTOCTOG	TOGETATEGY	CATOGGAATG	ATAGTOACOG	ACTOCOCCA
31101	TANCTACTOC	: CACTGGTAGC	TRECOCATIO	ACTHORAGA	OCCCATITIAT	ACACANANTO	GAMACTAGG	ACTABAGTAC	accactects	TOCATIOTAL .
	ATTICATIONCO	OTGACCATCG	AACCCGTAAC	TOMOTHER	CONSTANTA	TCTCTTTTAC	CTTTTGATCC	TOATITICATO	CCCCGAOGAA	ACCTACATT\
31201	ACACCACCTA	AACACTITICA	CCGTAGCAAC	TOOTCCAGOT	GREACTATTA	ATAATACTTC	CTTGCMACT	ANGITACIO	CAGCCTTOCO	TITICATICA
	TCTGCTGGAT	THOTOLANCT	OCCATCOTTG	ACCAGGTCCA	CACTGATAAT	TATTATCAAG	GAACCITTGA	TTTCAATOAC	CTCCCAACCC	NAMETANG .
31301	CARGECAATA	1 TOCANCTTAA	TOTACCAGGA	GCACTAARGA	TRIATTICTCA	NACAGACGC	CTTATACTTO	ATOTTACTTA	SCORMINGAL	GCTCANAAC :
	GITCCOTTAT	ACCITICAATT	ACATCGTCCT	CCTGATTCCT	AACTAAGAGT	THOTOTOCO	GAATATGAAC	TACARTCAAT	ACCCAARCTA	CCAOTTIN
31401	ACTAMITET	- MANCTADAN	CAGGGCCCTC	TTTTTATAMA	CTCAGCCCAC	AACTTOCATA	TTAACTACAR	CAMOOCCTT	PACTIONITA	CACCTICAN
	TEGATITAGA	TICHOATICE	GICCCGGGAG	ANANATATTE	GACTCGGGTG	THGAACCTAT	ANTTGATGIT	GTTTCCOOM	ATGAACAAT	GICGAAGTT
		Handlik								
31501	CAATTCCAAA	NAGCTTGAGG	TTAACCTAAG	CACTGCCANG	CONTRACTOR	TTGACCCTAC	AGCCATAGCC	ATTANTOCAG	GAGATOGGCT	TOMPTHON: F
	THIOGRAPH	THEODACTEC	AATTOGATTC	GTGACGGTTC	CCCNACTACA	AACTOCGATG	TOCCTATOCG	TAATTACGTC	CTCTACCCGA	ACTINANCEA
31601	TCACCTAATG	CACCAAACAC	AAATCCCCTC	ANAACAMAA	TYRECATOR	CCTAGAATTP	GATTCAAACA	AGGCTATOGE	TCCTAAACTA	COLACTICAT
	ACTOCATTAC	orocratoro:	TITACOCCAG	THEFTH	ANCCERTACE	GGATCTTAAA	CTAACTTTCT	TECGATACEA	ACCATITICAT	CCTTGACCTR
31701	TTAGTTTTGA	CACCACAGGT	OCCATTRONG	TAGGAAACAA	MATAATGAT	AAGCTAACTT	TITTAGACCAC	ACCAGCTCCA	TCTCCTAACT	GTACIACTAAA
	MICHANCE	GREGIOTICEA	COSTANTORC	Arccirrent	TTTATTACTÀ	TRUBATTION	ACACCTRAFIG	TOCTCGAGGF	ACAGGATTGA	CATCTGATTT
31801	TUCKGAGAAA	CATOCTANC	TCACTITION	CTTAACAAA	TOTAXCAGTO	MANTACTTGC	TACACTTRCA	OFFITTO COCTO	TTAAAOOCAO	THOGENICA
	ACONCICIENT	CTACGATTTO	AGTOLANCCA	GAATICITIT	ACACCCTICAG	TITATGAACG	ATCTCANGE	CAMACCONC	ANTIFICEGIE	AAACCGAGGT
31901	ATATCHCOM	CASTICIANG	TOCTOMET	ATTATAAGAT	THENCEANA	TERROTGETA	CTAMCAATT	CCTTCCTGGA	CCCARAATAT	T. AACTTTA
	TATACACCIT	GICARGITIE	ACCONGINGAA	TAATATTECTA	ACTOCITITE	ACCTCACGAT	CATTICITION	GRANDGACCT	OCCULTATA	ACCTICANAT
	-1	5								
32001	CHANTOCHCA	TETTACTOM	OCCACARGET	ATACAMACGE	TUTTOUT		TATICACITY			CHOCCANANG
٠	CTTACCICT	AGNATICACTT	CCCTCTCAGA	TATCHTACG	MCANGCTANA	TACTICATION	ATAGTCGAAT	ACCITITINGA	GROCCATTIT	GACOGITHIC

Figure 15T

32101	TANCATTOTC	AGTCAAGTTT	ACTTANACCES	MERCANANCT	AAACCTTGTAA	CACTAACCAT	TACACTARAC	GCTACACAGG	MACAGGAGA	CACAACTOTA
	ATTICTAACAG	TCAGTTCAAA	TGANTTINGC	TCKSTTTKA	TTTK2:ACATT	GICATINGTA	ATCHARTE	CCATGTGTCC	Trigicorci	CTCTTRACE :1'
32201	AGTGCATACT TCACGTATGA	CTATOTCATT GATACAGTAA	TTCATCCCTG	TRESTONEION ANDAGRES	ACAN TACAT TOTAXATITA	TATTACTTATA	TTTCTCACAT AMCCATTCTA	CCTCTTACAC GCAGAATGFC	TTTTTCATAC AAAAAGTATG	ATTRICICAN: TAACIBICITY:
32301	AATAAAGAAT	COTTTOTOTT	ATCHTECMC	CACAMTAN	TTCANTITION ANGTONIA	CHTFTAAAGT	NOTE ATTENT TENETANANA	CATTCAGTAG	TATAGCCCCA	CCACCACATA
32401	GCTTATACAG	-	CTTAATCMA		CCTAGTATTC		CHICCHICOCA	ACACACAGAG	TACACAGTCC	TTT: TECC: YE
	COMPATOTO	TACTGGCATO	GAATTAGETT	CACTOTOTO	GCATCATAAG	THYCACACTC	TANGO CANTO	TOTOTOTOTO	ATGTGTCAGG	ANAGRAGGO
32501	GCTGGCCTTA	_	-	_	TECHTAGATA	TTATATTCCA	CACCOTTICE	TOTOGRADOCA	AACOCTCATC	AGTOATATT
	CGACCGGAAT	TITICOTAGE	ATAGTACCCA	TIGICICIAL	AN:ANTCCAC	ATATAGGT	OTIGIC ANNUE	ACARCTOSOT	TTGCCAGTAG	TCACTATA
32601	ATAMETECE	-		ATGTCCCTGT	تديدها المتلاة		TOCTOTOCAA	CTYRECUSTIC	CTTAACOGC	GOTTANOTA:
	TATTTCAGGG	CCCCOTCGAG	TOANTHCANG	TACAGCGACA	CATTLEMEGRAC	TOGGICTOCG	ACGACAGGTT	GAACGCCANC	GAATTGCCCO	ככתכבווכבו.
						-#	Pett			
32701	ANGTOCACOC	CTACATOGGG	GTAGAGICAT	ANTEGRECAT	CAGGATAGGG		CHATRICATION GCAGCAGCGC	GCDANTAAAC	TOCHOCCOCC	OCCUCACO
	TTCABOTOCO	GATGTACCCC		CATCTCAGTA TTAGCACGTA	GICCTATCCC	RCCACCACGA	concences	COCHIMITIO	ACCIACOCCOG	CGCCGAGGCA
	Part									-
32801	CCTCCAGGAA	-	FACAACATOG CAOTOGICTC	CTCAGCGATG	ATTEGCACEG	CCCCACANCAR	ARGCINCIT	GTCCTCC068	CACAGCAGCG	CACCCTIGAT .
	GGACOTCCTT	ATOTTOTACC	GTCACCAGAG	GACTCCCTAC	TAAGCGTGTG	GCCCGTCGTA	TPCCCCCCCAA	CACCACCCC	GTOTOGTOGE	GTCCCACT"
			Z							
32901	TCACTTAKAT	CACCACACAGTA	ACTGCAGCAC	AGCACCACAA	TATTGHECAN	AATCCCACAG	TGCAMGCGC	TOTATECAAA	CCTCATGGGG	COCACCACAG
	ACTCAATTTA	GICOTOTCAT	TOACOTCOTO	recreeners	ATANCANOTT	TTAGGGTGTC	ACCITICORD	ACATAGOTTT	CONGINCOCC	CCCTCOTICTY
33001	ACCCACOTO	GCCATCATAC	CACAAGCGCA	CCTACATTAA	GTAGGGAACCC	CTCATABACA	CRETRIGACAT	AAACATTACC	TCTTTTGGCA	TOTACTAATT
	THOOOTOCAC	COSTAGTATO	GIGTTCGCGF	CCATCTAATT	CACCGCTGGG	CACTATTICT	CCCACCTIGTA	THOTAATOG	AGAMAACCOT	ACAACATTAA
		Kprd		,						lig 7
33101	CACCACCTCC	D		TAAACCICIG ATTAAACATG	GCGCCATYCA	CCACCATCCT	NACCAGCTO	GCCANACCT	acccaccacc	TATACACTY 2
	GTGGTGGAGG	GCCATGGTAT		ATTICCACAC TAATITICIAC	CGCGSTACGT	GOTTOGTACCA	TTTOTTOTAC	COCTITITICA	cooccecco	ATATOTCAC:
	Ē;							Engly		
33201	ACCOMACCOG	GACTOGAACA	ATGACAGITIO	AGAGCCCAGG	ACTICITABLE	ATGGATCATC	NTCACTCGTCA	TCATATCAAT	GTTGGCACAA	CACAROCACA
	Teceprooce	CTOACCTTOT	TACTOTOACC	1CTCGGGTCC	TONOCATTOO	TACCTAGTAG	TACCAGCAGT	ACTATAGETA	CACCOROTT	CTUTTICOTOT
	•									P.51
33301	COTOCATACA	CTRCCTCAGG	ATTACANGET	CCTCCCCCOTT	TACARCCATA	TCCCAGGGAA	CACCCATTC	CTCAATCAGC	OTAMATCCCA	CACTICAGIR
	OCACOTATOF	GANGGAGTCC	TAATGTTCCA	CHARTCACOCA	ATCTINGTAT	ACCEPTO	CTTCCCTANG	GACTTAGTO	CATTITACOOP	GROACCITCI:
33401	ANGACCTOC	ACCSTANCTCA	COTTOTOCAT	TOTOMOTO	THACATHOOD	CICAGCAGCTAT	ATGATECTEC		COCCOGNITIC	TOTOTOWN
	TTCTOGNOCO	TOCATTOAGT	OCANCALGTA	ACASTITICAC	ANTOTANGEC	משמשמשב	TACTACASAGG	TCATACCATC	OCOCCCANO	ACAGAGETE
33501	OCAGOTACAC	GATCCCTACT	GTACGRAFTS	CTACTONTACA	ACCIOAGATOS	TOTAL STATES	ACTOTOTO	CANATOGNAC	CCCCCACOTA	CHCATATTR.
	CCTCCATCTO	CTAGGGATAGA	CATAXCTICAC	CCGCCTCTGT	TOCCTCTACC	ACAACCAACA	TCACAGTACG	GTTTACCTTO	CCCCTCCAT	CACTATAAN'

5

33601	CTGAACCAAA	ACCAGGICACG	GOCGTGACAA	ACASATCTIC	טאייאינינישנ	TURCUCULAR (GATCOCTCTG			CACTCTCTTA
	GACTICOTIT	TOCICCACGC	CCCCACTEST	TCTC:TAGACG	ころいろのかいころ	APTOCKOUNT	CTANCANGAG	ACATCATCAA	CATCATATAG	CTOACAGART
33701	AAGCATCCAG	GCGCCCCCT0	OCTICONAL	CTATICTIANG	TCCTTYCATGG	טטטטטטטטט	TGATAACATC		_	CACCCAGCC
	Treoragore	COCCOCCCCAC	CGAAGCCCAA	GATACATTRE	AGGAAGTACG	COCCCACGGG	ACTAITECTAG	OTCOTOGCOT	CTTATTCGGT	GTGG(:TCG:II'
33801	ACCTACACAT	TCGTTCTCCC	ACTCACACAC	COCAMANACC	נאנאענאנאבעני	CHACHACCAT	GITTELLE	TTATTCCAAA	AGATTARCEA	AAACCT117AA
	TCCATCTGTA	ROCANGACOC	TCAGTCTGTG	CCCTCCTCCC	CUTTCTURAL	CTICTICGTA	CANAMARA	AATAAGGTTT	TCTAATAGGT	THEORETT
	E S	2								
33901	ATCANCATCT	ATTANCTORA	COCOCICCCC	PCCGGTGGCG	TYGHTCANACT	CTACACCCAA	AGAACAGATA	ATCOCATTO	TANGATOTTO	CACANTEGET
	TACTTCTAGA	TAATTCACTT	accenacae	ACCCCACCC	ACCAGITICA	GATGTCGGTT	TCTTGTCTAT	TACCGTAMAC	ATTCTACAAC	GTOTT'ACCC.
34001	TECAMAGGE	AAACOOCCCT	CACGTCCAAG	TCCACCTAAA	CCCTANACCC	TTCAGGGTGA	ATCTCCTCTA	TAMCAFTCC	ACCACCTICA	ACCATCCCCA
	AGOTTITICES	THUCCOOCH	OTGCAGGTTC	ACCTOCATTT	CCCATTICAGO	ANGTECECACT	TAGAGGAGAT	ATTTOTAGG	TCGTGGMGF	TOCTACOCCT
34101	ANTANTICIC	ATCTCOCCAC	CTTCTCMJTA	TATCTCTAAG	CANATCCCGA	ATATTAAGTC	CCCCCCATTGT	AAAATCTGC	Techandood	CCTCCACCTT
	TTATTANGAG	TAGAGCCOTO	GAAGACITAT	ATAGAGATIC	GTTTAGGGCT	TATAATTCAG	OCCURATANCA	TITITAGACG	Addrescood	GCAGGTGGAA
14201	CACCTCAG	CACCGAATCA	TGATTGCAAA	AATTCAGGTT	CCTCACAGAC	CTCTATAMEN	TTCANAGED	GAACATTAAC	AVAVATACEG	CONTCCCOTA
	GPCOGROTTC	_	ACTAACCTET	TTANGTCCAA	GCAGTGTCTO	GACATATTCT	MATTITOC	CTTCTAATTG	THTTATOOC	GCTACKCCAT
34301	COTCCCTICG	CAGGGCCAGC	TGAACATAAT	CGTGCAGGTC	TOCACOGACC	AGCGCGGGCGA	CTICCCCCCC	ACCUANCEATE	ACANAGGAC	CCACACTGAT
1	CCAGGGAAGC	greceastes.	ACTIGINATIA	GCACGTCCAG	ACCITOCCTOO	TCGCGCCGGT	GAAGGGGCGG	recredetac	TCHTTTCHO	GGTGTGACTA
					Handi					
19401	TATERCACIC		ATACTOGGIO CTATOCTARS	CACCETAGE	CCCATCTAAG	CTTGTTGCAT	OCCCCCCA1	ATHAMATOCA	Aggreeract	CAMMATCI
		-	GATACGATTG	GTCGCATCGG	COCTACATTC	GANCANCOTA	CCCCCCCCTA	TATTETACOF	TCCACGACGA	GITTITIAG.
34501	COCAAACCCT	COCCCANANA	NGARAGCACA	TCOTAGTCAT	CATHCATGCAG	ATAMAGGCAG	OTARGCTCCG	GNCCACCAC	AGANANAGAC	ACCAPITITIO
	CCGTTTCGGA	_	remicons	ACCATCAGTA	CCAGTACGTC	TATTICCOTC	CATTCGAGGC	crreateora	retition.	TOSTANANG
34601	TCTCAAACAT	OPETGCOCOF	TTCTGCATAA	ACACAMATA	ANATAACAAA	ANACATITA	AACATTAGAA	GOCHGICTTA	CAACACCAAA	AACAACCCTT
	AGAGITTOTA		AAGACCTATT	TGROTETTAT	TEATHGITT	TITICINANT	TTGTWATCTT	COCHCACACAT	orionecrit	TICHICCON
34701	ATMOCATAL	GACCONCTAC	GCCCATGCCG	OCCITUACCUI	ANNANACTO	OTCACCOTGA	TTAMANGCA	CCACCCACAG	CICCICGGIC	ATGTCCCONG
ı	TATTCOTATT	CTOCCTOATO	CCCCTACCCC	COCACTOCICA	TITITITICAC	CAGTCACACT	ANTITUTOR	GOTGOCTGTC	GAGGAGGCCAG	TALMARCTC
34801	TCATAATOTA	AGACTICGOTA	AACACATCAG	GITCATICAC	ATCOSTCAGE	GCTAANANGC	GACCGANATA	OCCCCCCCCCA	ATACATACCC	CENTRY CTAG
	AGTATTACAT	-	TIGICIANT	CAACTAACTO	TACCCAGTCA	CGATTITICG	CTGGCTTTAT	COCOCCCCCT	TATOTATOOG	CGICCCCATC
10901	ACACANCATT	. ACAGCCCCCA	TAGGAGGTAT	AACAANATTA	ATAGGAGAGA	ANNCACATA	AACACCTUAA	AMCCCTCT	GCCTARGCAA	AATAGCACCC
	ACTOTIOTAL	tercooper.	ATCCTCCATA	TICHTIMI	TATCCTCT	THINGTHE	TTGTGGACTT	THIOCOACCA	COONTCCUT	Trancordor
35001	TECCOCTECA	GUNCANCATA	CAGCGCTTCC	ACACCOCCAG	CCATAACAGT	CACCETACE	ACTANAMANG	AAAACCTATT	MANANACAC	CACTOTALM:
	ACCOCCOACCE	CHIGHISTAT	CTCCCGANGO	TOTOCOCCOTO	OGTATTOTCA	OFFICEAMTOG	1CATTETEC	TTTTCCATA	नाममाठाठ	GIOAGCTOTO
15101	GOCACCAGCT	r CHATCAGTCA	CAGTGTANA	MOONCE/MO	TOCAGAGOGA	GTATATATA	CACTAAAAA	TORCOTANCO	GITAMOTCC	ACALANARIA
	CCOTOGTCGA	_	GTCACATITI	Trecedente	ACGRETECICE	CATATATATE	CICATITITE	ACTOCATION	CAATITICAGG	101111111111111111111111111111111111111
15201	CCCAGAAAAC	COCACOCOAA	CCTACOCCCA	CANACCANAG	CCANANACC	CACACTICC	TCAMTCGTC	ACTROCOLL	TCCCACGITA	CULCACITICA
	DESTRICTION	1 OCOTOCOCTT	CCATCCCCCT	CTTRACTIFIC	OCTTTTT00	GICTIGAAGG	ACTITARICAG	TCAACCEAAA	ACCOTOCAAT	GCMUTGAAGG

Figure 15V

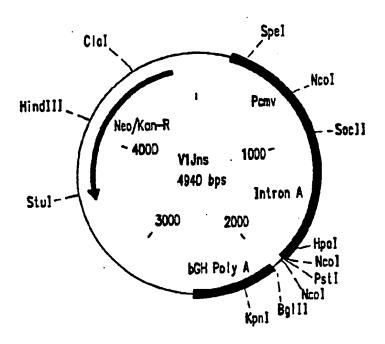
35301	CATHITARGA				Trenceta	AACCTACGTC ACCTGCCCCG		TTCCCACGCC	CCCCCCACG	TCACAMETE
•	CIMMATICA	ALISTANTI	According	AICTICANIC MASSACIANI	Jakerna	LOS			merce con the	
35401	CACCCCTCA	TTATCATATT AATACTATAA	GCTTCAATC	CANANTANGG	TATATTATTG	ARCHACATTA	tus	CCTMCACGCT	COCHAGOCTO	CTACCOCATA
15501	CCCATTATGA	THETTETEGE	TTCCGGGGGG		CCGCCTTGCA	CCCATCCTC	TCCAGGCAGG AGGTCCGTCC	TAGATUACGA	CCATCAGGGA	CACCTICAAO
35601	CONCOLUTE	GGCCAGGAAC	CCTTAAAAAGG	CCCCCOTTCCT GCCCCACGA	CCCCAAAAG	CATACACTAC	GCCCCCTGA	CCACCATCAC	ANAMATECAL	CCACTTCAGE
15701	CHECACTOCTA	MCCCCACAG	GACTATAAAG	NTACCAGGCG TATAGGTCCGC	AAAGTGGGCTG	CHICANGOCA	CCACCCCACA	CCTUTTCCGA	CCCTGCCGCT	TACCEGATAC ATGGCCTAT
35801	CTURCCUCCT	TTCTCCCTTC ANGAGGGAAG	CCCTTCGCAC	GCGCTTTCTC CCCGANAGAG	ATARCTCACG TATCGAGTOC	CTCTARGTAT	CTCANTICOS	TOTAGGTCOT ACATCCAGCA	PCCCPCCAAG ACCCAGOTIC	CTCGGCTTGT.
35901	TOCACOAACC	CCCCGTTCAG	CCCGACCGCT	CCCCCANTAG	CCCNTTCATA	CONCINGACI	CCAACCCGGF	AAGACACGAC	TTATCOCCAC ANTAOCGGTO	TOOCAGCAG".
36001	CACTOSTAAC	AGGATTAGCA	CTCGCTCCAT	TOTAGGGGGT ACATCCGCCA	CCATCTCACA	TETTOMOTO AGMCTTCAC	GTOGCCTAAC	TACOGCTACA	CTAGANGIAC	ACTATTICOT TCATAAACCA
36101	ATCTOCGCTC	TOCTOANGCC ACGACTTCGG	AGITINCCTTC TCANTOGAND	CCTTTTTCTC	THESTAGETE AACCATEGAG	THGATCEGGC AACTAGGCCG	AAACAAACCA	CCCCTGGTAG	CCCACCAAA	TTTGTTTGC.
36201	AGENGEAGAT	TACGCGCAGA	AAAAAAGGAT TTTTTTCCTA	CTCANGANGA	ACCITITGATE	TTTTCTACGO	CCAGACTICO	TCAGTGGAAC AGTCACCTTG	GAMMACTICAC	GTT-MOGGN! CAATTCCCT!
36301	AMCCAGIAC	ACATTATCAA	MAGGATOTT	CACCTAGATO	CTTTTAAATC	ANTCHANGE	ATATATGAGT TATATACTCA	AAACTTGGTC	TCACAGTTAC ACTOTCAATO	CARTCCTTA
36401	TCAOTOROOC AGTCACTCCO	ACCTATCTCA TOGATAGAGT	OCCUPACTORC COCTAGACAG	TATTTCGTTC ATAMACCAAG	ATCCATAGTT TAGGTATCAA	GCCTOACTCC CTGACTGAGO	CCCTCCTCTA	GATAACTACG CTATTGATGC	ATACGGGAGG TATGCCCTCC	GCTTACCATY CCANTAC
36501	TOOCCCANT	CCHOCANTOR COACGITACT	PACCOCCAGA ATGCCCTCT	CCCACCCTCA	CCGCCTCCAG	AFFFAFCAGC TAAATAGTCC	ANTANACCAG TTATTTGGTC	CCACCCCCAA COTCCCCCT	GOGCCOAGCG	CACANATION
36601	CCTCCACTT	TATCCOCCTC ATAGGCGGAG	CATCCAGTCT	ATTAATTCTT TAATTAACAA	GCCCCCANGC	TATANTTAANT ATCTCATTCA	AGTTCGCCAG TCAAGCAGTC	TTAATAGTITT AATTATCAAA	GCGCAACGTT	CANCOGTANG
36701	CTACAGGEAT	CONCENCIAL	COCTCGTCGT OCCAOCAGEA	TTOOTATGOC AACCATACCG	TTCATTCAGC AAGTAAGTCG	TCCGGTTCCC AGGCCAAGGG	AACCATCAAG TTGCTAGTTC	GCGAOTTACA CGCTCAATOT	TCATCCCCCA ACTAGGGGGGT	ACAACACGTT
36801	AAAAGCGGTT TTTTCGCCAA	ACTCCTTCO TCGAGGAAGC	Greetecart	2 -	ACTAAGTTISG	CCCCAGRATT	ATCACTCATG	CATACCOTC	CACTOCATAA	TICTCTTACT AAGAGAATGA
36901	GTCATGCCAT CAGTACGGTA	CCCTAAGATG GCCATTCTAC	CHTTECTOTO	ACTRICATEMENT TRACCACTICA	TOPOLICOL	CACTANGACT	CTTATCACAT	ACCCCCTCC	CTCMCGAGA	ACCOCCOCA

figure 15W

PMRKAdSgag MER682

37001 37101 37201 37301	CMCACGODA FINATMOCGCO CCACATARCA GAACTITANA AGTINITICAL ATTRIAMANC GITCTHIGGG GCGANAGCTE TCAGGATCT TACRICIANA GITGECCCT ATTAIGACC GCTOTATCH CITTLANATT TCAGGATAG TANCCITITG CARGAGGCC CCCTTTTORG AGTICCTORG ATTGAGATT TCAGGATAT CATTRIANATA CATTAIGATTA CATTRIAMANT CATTRIAMANA CATTRIAGACC AGTITTHOCC TTCGGTTTTA CANANTISTA CATAGGATA AGCOCACA GATTHIGAC TAGGATTA AGAMITISTA CANANTISTA CATAGATATA CAGGAGTAT TCAGTTTA TCAGTATA AGGATTAT CAGGATTAT CAGGAGTATT CAGGAGTATT CAGGAGTATT CAGGAGTATT CAGGAGTATT CAGGAGTATT CAGGAGTATTA CAGGAGTATT CAGGAGTATT CAGGAGTATT CAGGAGTATT CAGGAGTATT CAGGAGTATTA CAGGATTATTA CAGGATATATA CAGGAGTATA CAGGAGTATTA CAGGAGTATA CAGGAGTA CAGGAGTA CAGGAGTATA CAGGAGTATA CAGGAGTATA CAGGAGTATA CAGGAGTA	FANTACCOCO ATTATOGCOC TCGATITANC ACCTRCATO AGGINATANG ATTIGATOC ATTIGATOT TAANCTTACA	CCACATARCA GUTGTATCAT CCACTCATG GUTGAGCACT GUTGAGCACT GUTGAGACA ATTRAGANA TANNTCTTTT	GOOD FINATINGCOCO CCACATARCA GAACTITANA AGTINITEATE ATTRIBANC GITCTATOGO GCONNACTE TCAGGATOT TACRETEATE CCCT ATTATOGOCO CCACATACAT CITELANATT TCAGGAGAS TANCCITITO CAGGAGCC COCTITIONG AGTICCTAGA ATGGGAGAAT CAGT TCGATTANC CGACTCGING ACTAMINITA TCATRIBANTA CITITALITIT CACAMICIALA AGACATAGA AGCINTANC CTAGGAGACT AGAMINITATA CANANTISTA CANANTISTA CATAMINITATA CAGGAGTATA TCAGGAGTATA TCAGGAGTATA TCAGGAGTATA AGAGATATA AGAGATATA CAGGAGTATA CAGGAGTATA AGAGATATA CAGGAGTATA CAGGAGTATA AGAGATATA CAGGAGTATA CAGGAGTATA AGAGATATA CAGGAGTATA CAGGAGTATA CAGGAGTATA CAGGAGTATA CAGGAGTATA AGAGATATATA CAGGAGTATA AGAGATATATA CAGGAGTATA CAGGAGTATA CAGGAGTATA AGAGATATATA CAGGAGTATA AGAGATATATA CAGGAGTATA AGAGATATATA CAGGAGTATA AGAGATATATA CAGGAGTATATA CAGGAGTATATA CAGGAGTATATA AGAGATATATA CAGGAGTATATA CAGGAGTATATA AGAGATATATA CAGGAGTATATA CAGGAGTATA CAGGAGTATATA CAGGAGTATA CAGGAGTATATA CAGGAGTATATA CAGGAGTATA CAGGAGTATA CAGGAGTATATA CAGGAGTATA C	AGTICTUTATION TEACGACTANI ACAMENISTA ACAMENISTA ACAMENISTA ACAMENISTA TECECCAMENIC	ATTGAMMIC TANCCITTING CANANTAMA GANGAMANA GANGAMMA CTCATTITI GCCTGTAMG	CAACAAACCC CACTAVCCTT CACTATCCTA CAATATATA CACCAAAAAAA GACCTTTTCA GACCTTTTCA	GCGAAAACTE CCCTTTTCAG ACCCCACTE GAAGCCATTTA CTTCCTAAAT GCCACCTGAC	TCAAGGATCT AGTTCCTAGA CAAAACACG GTTTTTGTCC TCAGGGTTTTGTCC AGTCCCATA GTCTAAGAAA CAGATTCTTT	ATCHECTOTA ATCCANANT TTCCCTTTTA TCCCTCATE: ACAGAGTALT
37401	CATGACATTA	ACCTATAAAA TOJATATITT	ATACCCCATA TATCCCCATA	CATCACATTA ACCTATAMA ATAGACGTAT CACGAGGCC TTTCGTCTTC ANGACTTGOA TYCGMATTCT TAAT (SEQ ID NO: 27) GTACTOTAAT TEGATATTT TATCCCCATA GTGCTCCXGG AMGGAGANG TYCTTAACCT AGGCTAAAA ATTA (SEQ ID NO: 28)	TTICGICTIC	ANGANTICA TYCE THETTAMEET AGG	TI CGANTICT AGCCTTAAGA	TRAT (SEQ ATTA (SEQ	ID NO: 27) ID NO: 28)	

Figure 15X



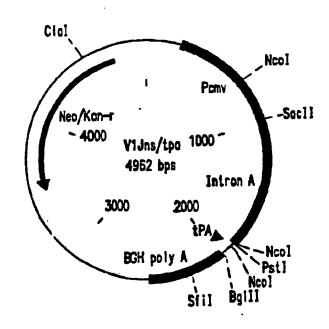


FIGURE 16

AGATC1 ACCATGGCCCCCATCTCCCC Bg/11 MetaloProlieSerPr 1	CATTGAGACTGTGCCTGTGAAGCTG OTTEGTUTHTVOTProVotLysLeul 10	AAGCCTGGCATGGATGGCCCCAAGGTGAA LysProGTyMetAspGTyProLysVoTLy 20
		TGAGATGGAGAAGGAGGGCAAAATCTCCA rG!uMelG!uLysG!uG!yLys!!eSerL 50
		AGGACTCCACCAAGTGGAGGAAGCTGGTG ysAspSerThrlysTrpArglysleuVol 70
GACTTCAGGGAGCTGAACAAGAGGAC AspPheArgGTuLeuAsnLysArgTt 80	CCCAGGACTTCTGGGAGGTGCAGCTG nrGInAspPheTrpGIuVoIGInLeu 90	GGCATCCCCCACCCCGCTGGCCTGAAGAA GlylleProHisProAloGlyLeuLysLy 100
GAAGAAGTCTGTGACTGTGCTGGCTG slyslysSerVolThrVolLeu <u>Alo</u> \ 110	GTGGGGGATGCCTACTTCTCTGTGCC VolG1yAspA1oTyrPheSerVolPr 120	CCTGGATGAGGACTTCAGGAAGTACACTG oLeuAspGTuAspPheArgLysTyrThrA 130
CCTTCACCATCCCCTCCATCAACAAT toPheTnrlieProSerileAsnAsn	TGASACCCCTGGCATCACGTACCAGT BluThrProGlylleArgTyrGinTy 140	ACAATGTGCTGCCCCAGGGCTGGAAGGGC yrAsnVolLeuProGInGIyTrpLysGIy 150
		AAGCAGAACCCTGACATTGTGATCTACCA LysGinAsnProAsplieVoilleTyrGi 180
		GACCAAGATTGAGGAGCTGAGGCAGCACC gThrLysIleGluGluLeuArgGlnHisL 210
TCCTGAGGTGGGGCCTGACCACCCC euLeuArgTrpGlyLeuThrThrPro	IGACAAGAAGCACCAGAAGGAGCCCC DASplyslysHisGInlysGIuProP 220	CCTTCCTGTGGATGGGCTATGAGCTGCAC roPheLeuTrpMetGTyTyrGTuLeuHis 230
DDCGACAGTGGACTGTGCAGCCCA ProAsplysTrpThrVoIGInProi 240	TTGTGCTGCCTGAGAAGGACTCCTGG 1eValleuProGlulysAspSerTrp 250	ACTGTGAATGACATCCAGAAGCTGGTGGG ThrVoIAsnAspIIeGInLysLeuVoIGI 260
		GTGCAAGCTGCTGAGGGGCACCAAGGCCC euCysLysLeuLeuArgGTyThrLysATal 290

FIGURE 17A

GCCCTGTACTATCACCCCTCCAAGGACCTGATTGCTGACATCCAGAAGCAGGGCCAGGGCCAGTGGACCTACCAAATCTA GlyVolTyrTyrAspProSerLysAspLeulleAloGlulleGlnLysGlnGlyGlnGlyGlnTrpThrTyrGlnlleTy 320 330 340

CCADGAGCCCTTCAAGAACCTGAAGACTGGCAAGTATGCCAGGATGAGGGGGGCCCCACACCAATGATGTGAAGCAGCTGA rGInGluProPheLysAsnLeuLysThrGiyLysTyrAlgArgMelArgGlyAlgHisThrAsnAspVoilysGInLeuT 350 350 370

CTC4GGCTGTGCAGAAGATCACCACTGAGTCCATTGTGATCTGGGGCAAGACCCCCAAGTTCAAGCTGCCCATCCAGAAG hrGluAloVolGinLyslleThrThrGluSerlleVollleTrpGlyLysThrProLysPheLysLeuProlleGinLys 380 390

GGTGAAGCTGTGGTACCAGCTGGAGAAGCAGCCCCATTGTGGGGGGCTGAGACCTTCTATGTGGCTGGGGCTGCCAACAGGG uVollysLeuTrpTyrCinLeuGiuLysGiuProlleVolGlyAloGiuThrPheTyrVolAloGlyAloAloAsnArgG 430 440 450

AAGACTGCCCTCCAGGCCATCTACCTGGCCCTCCAGGACTCTGGCCTGGAGGTGAACATTGTGACTGCCTCCCAGTATGC LysThr AloLeuGInAlolleTyrLeuAloLeuGInAspSerGlyLeuGluVolAsnIleVolThr AloSerGInTyrAl 480 490 500

CCTGGGCATCATCCAGGCCCAGCCTGATCAGTCTGAGTCTGAGCTGCTGAACCAGATCATTGAGCAGCTGATCAAGAAGG aleuGiyiieIieGinAioGinProAspGinSerGiuSerGiuLeuVolAsnGinIieIieGiuGinLeuIieLysLysG 510 520 530

ACAAGGTGTACCTGCCCTGCCTGCCCCACAAGCCCATTGGGGGCAATGAGCAGGTGGACAAGCTGGTGTCTGCTGGC
lulysvoltyrleualotrpvolproalohislysglylleglyglyAsnGluGlnVolAsplysleuvolSerAlogly
540
550

ATCAGGAACGTGCTGTTCCTGGATGGCATTGACAACGCCCCAGGATGAGCATGAGAAGTACCACTCCAACTGGAGGGCTAT
11eArgLysVolleuPheleuAspGlyI1eAspLysAloGlnAspGluHisGluLysTyrHisSerAsnTrpArgAloMe
560 570 580

FIGURE 17B

CGCCTCTGACTTCAACCTGCCCCTGTGGTGGCTAACGAGATTGTGCCCTCCTGTGACAAGTGCCAGCTGAAGCCCCAGG tAloSerAspPheAsnLeuProProVolVolAloLysGiuIleVolAloSerCysAspLysCysGinLeuLysGiyGiuA 590 600 610

CCATGCATGGGCAGGTGGACTGCTCCCCTGGCATCTGGCAGGTGACCCACCTGGAGGGCAAGGTGATCCTGGTG IOMetHisGlyGlnVolAspCysSerProGlylleTrpGlnLeuAloCysThrHisLeuGluGlyLysVollleLeuVol 620 630

GCTGTGCATGTGGCCTCCGGCTACATTGAGGCTGAGGTGATCCCTGCTGAGACAGCCCAGGAGACTGCCTACTTCCTGCT AlovalHisValAlaSerGlyTyrlleGluAlaGluVallleProAlaGluThrGlyGlnGluThrAlaTyrPheLeuLe 640 650 660

GAAGCTGGCTGGCAGGTGGCCTGTGAAGACCATCCACACTGCCAATGGCTCCAACTTCACTGGGGCCACAGTGAGGGCTG

LLysLeuAloGlyArgTrpProVolLysThrIleHisThrAloAsnGlySerAsnPheThrGlyAloThrVolArgAloA

680

690

CCTGCTGGTGGGCTGGCATCAAGCAGGAGTTTGGCATCCCCTACAACCCCCAGTCCCAGGGGGTGGTGGCCTCCATGAAC IoCysTrpTrpAloGly!leLysGInGluPheGly!leProTyrAsnProGInSerGInGlyVolVolAIoSerMelAsn 700 710

AAGGAGCTGAAGAAGATCATTGGGCAGGTGAGGGACCAGGCTGAGCACCTGAAGACAGCTGTGCAGATGGCTGTGTTCAT LysGluLeulysLyslielleGlyGInVolArgAspGInAloGluHisLeulysThrAloVolGlnMeiAloVolPhell 720 730 740

CCACAACTICAAGAGGAAGGGGGCCATCGGGGGCTACTCCGCTGGGGAGAGGATTGTGGACATCATTGCCACAGACATCC
eHisAsnPheLysArgLysGiyGiylieGiyGiyTyrSerAloGiyGiuArglieVolAsplieIleAloThrAsplieG
750
760
770

AGACCAAGGAGCTCCAGAAGCAGATCACCAAGATCCAGAACTTCAGGGTGTACTACAGGGACTCCAGGAACCCCCTGTGG
InThrLysGluLeuGInLysGIn!ieThrLysI!eGinAsnPheArgVolTyrTyrArgAspSerArgAsnProLeuTrp
780 790

AAGGCCCTGCCAAGCTGCTGTGGAAGGCGGAGGCGGTGTGGTGATCCAGGACAACTCTGACATCAAGCTGGTGCCCAG LysGiyProAiolysLeuLeuTrpLysGiyGiuGiyAioVoiVoiIieGinAspAsnSerAspIieLysVoiVoiProAr 800 820

AAAGCCCGGCAGATC" (SEQ ID NO: 3)
Xx Bq/I 1 (SEQ ID NO: 4)

FIGURE 17C

GATCACCATGGATGAAGAGAGGCTCTGCTGCTGCTGCTGTGTGCAGCAGCATCTTGCTTTGC

Met AspAt Gall ys ArgGtyleuCysCysVolleuleuCysGtys 1 oVotPheVol SerP

-25

CCACCOMGATCTCCCCCCCATCTCCCCATTCAGACTGTGCAGCTGAAGCTGAACCTGCAAGCATGCC (within SEO 10 NO: 7)
RoSerGiulleSerAidProlleSerProlleCluthrVolProValLysLeutysProClyMetAspCly (within SEO 10 NO: 8)
-1 2 20

FIGURE 18

พา	- ATG GGT GGC AAG TGG TCA AAA CGT AGT GTG CCT GGA TGG TCT -42	
OPT	- ÁTG GÓC GÓC ÁÁG TGG TĆC ÁÁG AGG TCC GTG CĆC GÓC TGG TCC M G G K W S K R S V P G W S -14	
WT	- ACT GTA AGG GAA AGA ATG AGA CGA GCT GAG CCA GCA GAT -84	
OPT	- ACC GTG AGG GAG AGG ATG AGG AGG GCC GAG CCC GCC GCC GAC T V R E R M R R A E P A A D -28	
WT	- AGG GTG AGA CGA ACT GAG CCA GCA GCA GTA GGG GTG GGA GCA -126	
OPT	- AGG GTG AGG AGG ACC GAG CCC GCC GCC GTG GGC GTG GGC GCC R V R R T E P A A V G V G A -42	
WT	- GTA TCT CGA GAC CTG GAA AAA CAT GGA GCA ATC ACA AGT AGC -168	
OPT	- GTG TCC AGG GÁC CTG GAG ÁÁG CÁC GGC GCC ÁTC ÁCC TCC TCC V S R D L E K H G A 1 T S S -56	
ਘਾ	- AAT ACA GCA GCT ACC AAT GCT GAT TGT GCC TGG CTA GAA GCA -210	
OPT	- ÁÁC ÁCC GCC ÁCC ÁÁC GCC GÁC TGC GCC TGG CTG GÁG GCC N T A A T N A D C A W L E A -70.	
WT	- CAA GAG GAT GAG GAA GTG GGT TTT CCA GTC AGA CCT CAG GTA -252	
OPT	- CÁG GÁG GÁC GÁG GÁG GTG GGC TTC CCC GTG ÁGG CCC CÁG GTG Q E D E E V G F P V R P Q V -84	
WT	- CCT TTA AGA CCA ATG ACT TAC AAG GGA GCT GTA GAT CTT AGC -294	
OPT	- CCC CTG ÁGG CCC ÁTG ÁCC TÁC ÁÁG GGC GCC GTG GÁC CTG TCC P L R P M T Y K G A V D L S -98	
WT	- CAC TIT TTA AAA GAA AAG GGG GGA CTG GAA GGG CTA ATT CAC -336	
OPT	- CÁC TÍC CTG AÁG GÁG ÁÁG GÉC GÉC CTG GÁG GÉC CTG ÁTC CÁC H F L K E K G G L E G L I H -112	
WT	- TCA CAG AAA AGA CAA GAT ATC CTT GAT CTG TGG GTC TAC CAC -378	,
OPT	- TCC CAG AAG AGG CAG GAC ATC CTG GAC CTG TGG GTG TAC CAC S Q K R Q D I L D L W V Y H -126	;
WT	- ACA CAA GGC TAC TTC CCT GAT TGG CAG AAC TAC ACA CCA GGG -420)
OPT	- ACC CAG GGC TAC TTC CCC GAC TGG CAG AAC TAC ACC CCC GGC T Q G Y F P D W Q N Y T P G -140)

FIGURE 19A

WT		CCA	11	111	11	11 .	11	-11	111	11	11	111		111	111	-462
OPT	•	ĊĊC P	GGC G		AGG R		CCC P	CTG L	ACC T	TTC	32 2 9	TGG W	TGC C	TTC F	AAG K	-154
WT		CTA	11	11	11	111	11	11	111	11	11		111	11	11	-504
OPT	•	ĊŤG L	ĠŤG V	CCC	GTG V	GAG	CCC	GAG	AAG	GTG	GAG	GAG	GCC A	aac N	GAG E	-168
WT		GGA	111	111	111	111	- 11	i	111	Н	111		111	11	-11	· 54 6
OPT	•	329 9	GÁG E	ÀÀC N	AAC N	ŤĠĊ C	CTG	CTG L	CAC H	CCC	ATG M	TCC S	CAG Q	CAC H	GGC G	-182
WT		ATA	111	111	11	111	111	-11	111	11	- 111	111	- 111	11	П	-588
OPT	•	ATC	GAG	GAC	ĊĊC P	GAG	AAG	GAS	GTG	CTG	GAG	TGG W	AGG R	TTC F	GAC D	-196
WT .	•	AGC	AAG		GCA		CAT		GTG	GCC	CGA	GAG	CTG	CAT	CCG	-630
OPT	•	TCC	AAG	ĊŤG	GCC A	TTC	CAC	CAC	GTG	ĞĊĊ	AGG	GÁG	CTG	CAC	CCC	-210
WT	•	GAG		TAC		GAC	TGC		(5	EQ I	D NC):30)				-651
DPT	•	GAC	TAC	TAC	AAG	GAC	TGC	TAA	((c	conta SEQ I	onet DNO	i wit : 10)	hin	SEC) ID NO): 9) -216

FIGURE 19B

VIJns/nef

CATGGGTCTTTT<u>CIGCAG</u>CCCCCCCCTCGAGAICTGCCACC ATG GGC GGC ANG TGG TCC ANG TGG TCC GTG CCC

Srf1 Bg111

CAC CCC GAG TAC TAC ANG GAC TGC TAA AGCCCGGGCACAGAICTGCCTTCTAGTTGCCAGC (SEQ 1D NU: 38)

H P E Y Y K D C * (contained within SEQ 1D NO: 10:

V1Jns/nef(G2A.LLAA)

Srf/ Bgllf
. . . CAC CCC GAG TAC TAC AAG GAC TGC TAA AGCCCGGGCACATCTGCCTGTGCCTTCTAATTGCCAGC (SEQ 1D NO: 39)
H P E Y Y K D C * (contained within SEQ 1D NO:14)

Vijns/tpanef & Vijns/tpanef(LLAA)

Psti Catgastettticigasteaccettatatetagateacce atg gat gca atg ang agg ctc tgc tgt gtg m D a m k r g l c c v

CTG CTG TGT GGA GCA GTC TTC GTT TCG CCC AGC $\frac{BgJIJ}{L}$ CC TCC AAG AGG TCC GTG CCC

CAC CCC GAG TAC TAC AAG GAC TGC TAA AGCCCGGGGCGGCTGCTGCCCTCTAGTTGCCAGC (SEQ ID NO: 40)

H P E Y Y K D C * (contained withon seq id no 16) BallI

FIGURE 20

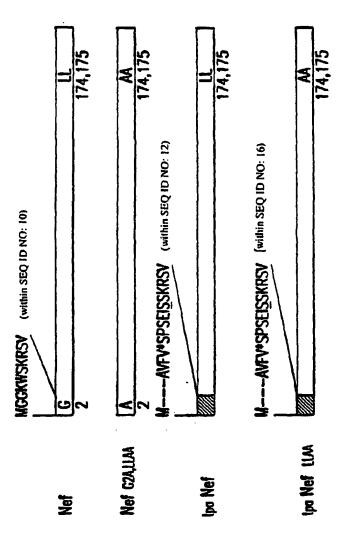


FIGURE 21

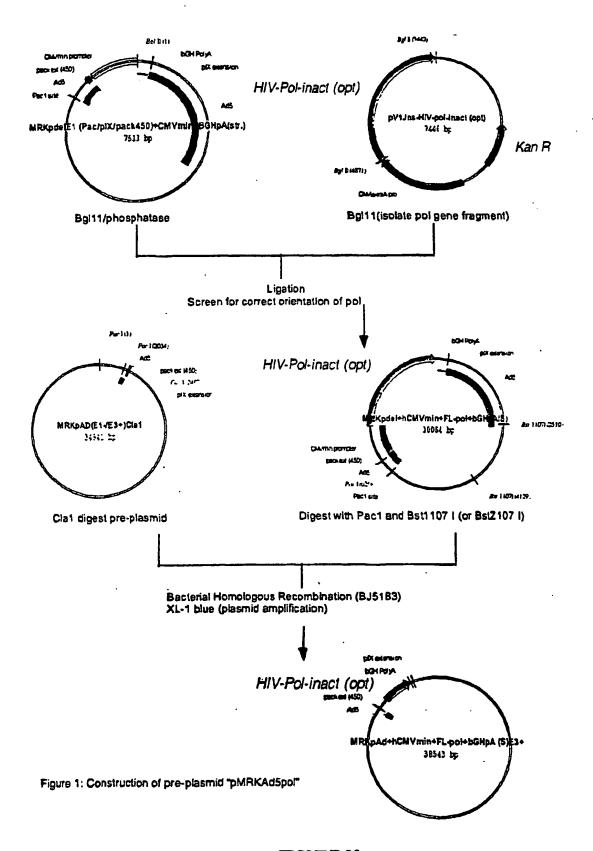
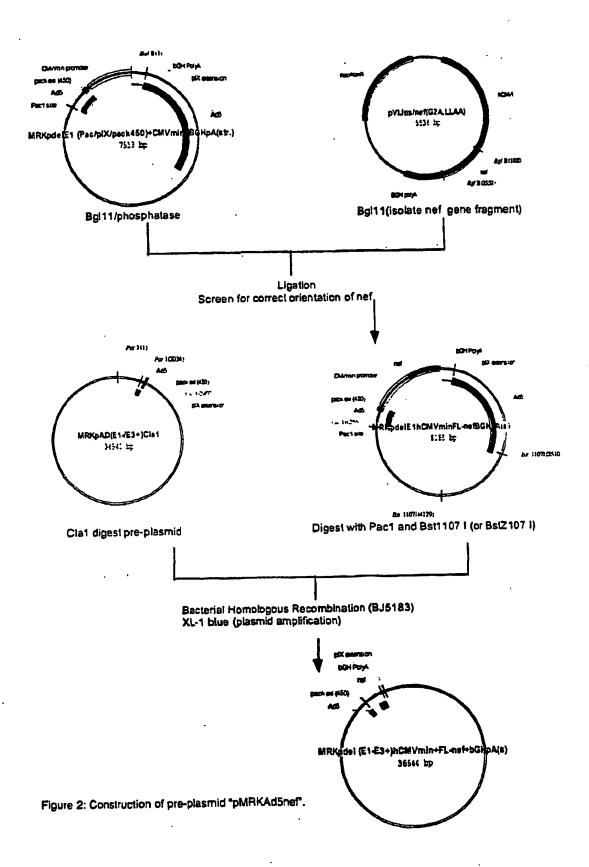
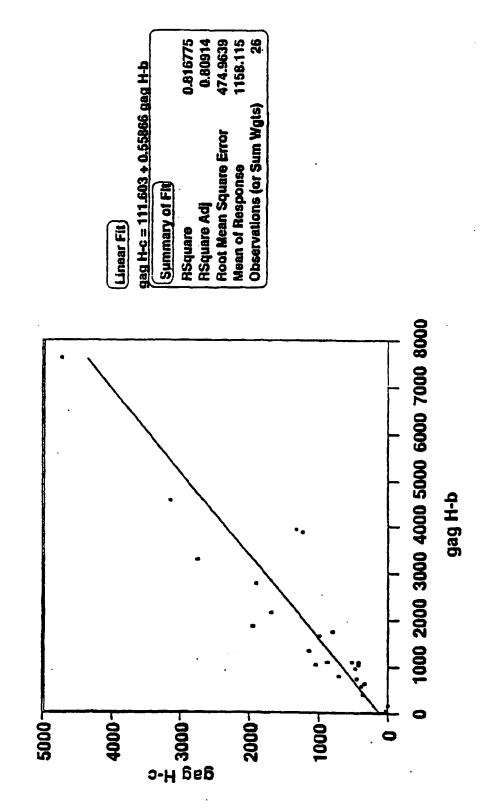


FIGURE 22



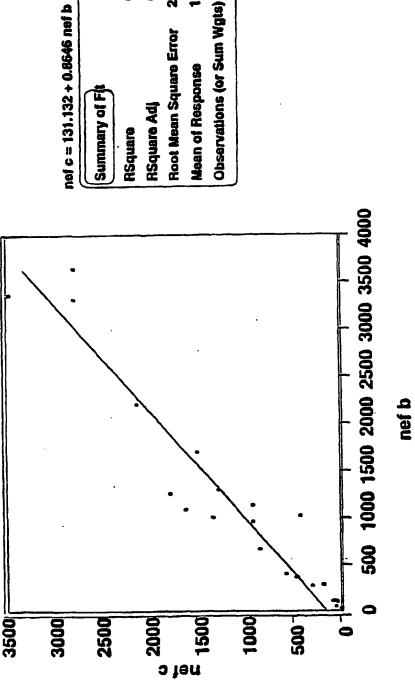
Comparison of Clade B vs. Clade C Anti-gag T Cell Responses in Clade B HIV-Infected Subjects



Comparison of Clade B vs. Clade C Anti-nef T Cell

Responses in Clade B HIV-Infected Subjects

nef c = 131.132 + 0.8646 nef b



EICHEE 72

1096.435

289.7718

R

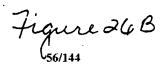
0.91685 0.91289

MRKAd5pol MER1062 (MRKAd5 Pre-Adenoviral Vector Containing the IA opt pol Coding Region)

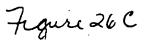
1	CATCATCAAT	AATATACCTT	ATTTTGGATT	GAAGCCAATA	TGATAATGAG
	GTAGTAGTTA	TTATATGGAA	TAAAACCTAA	CTTCGGTTAT	ACTATTACTC
51		TTGTGACGTG			
	CCCCACCTCA	AACACTGCAC	cececccccc	ACCCTTGCCC	CGCCCACTGC
101		GCGGAAGTGT			
		CGCCTTCACA			
151		TGGCAAAAGT			
	CGCTGCCTAC	ACCGTTTTCA	CTGCAAAAAC	CACACGCGGC	CACATGTGTC
201		TTTTCGCGCG			
		AAAAGCGCGC			
251		TAAGATTTGG			
		ATTCTAAACC			
301		GAATAATTTT			
	TCACTTTAGA	CTTATTAAAA	CACAATGAGT	ATCGCGCATT	ATAAACAGAT
351		GACTTTGACC			
	CCCGGCGCCC	CTGAAACTGG	CAAATGCACC	TCTGAGCGGG	TCCACAAAAA
401		TTCCGCGTTC			
		AAGGCGCAAG			
451		TCCATTGCAT			
	CGCCGGCGCT	AGGTAACGTA	TGCAACATAG	GTATAGTATT	ATACATGTAA
501		CATGTCCAAC			
		GTACAGGTTG			
551		TAGTAATCAA			
		ATCATTAGTT			
601		CGTTACATAA			
		GCAATGTATT			
651		CCCGCCCATT			
	6661166166	GGGCGGGTAA	CIGCAGITAT	TACTGCATAC	AAGGGTATCA
701	AACGCCAATA				
•	TTGCGGTTAT	CCCTGAAAGG	TAACTGCAGT	TACCCACCTC	ATAAATGCCA
751	AAACTGCCCA				
	TTTGACGGGT	GAACCGTCAT	GTAGTTCACA	TAGTATACGG	TTCATGCGGG
801	CCTATTGACG				
		AGTTACTGCC			
851	CATGACCTTA				
	GTACTGGAAT	ACCCTGAAAG	GATGAACCGT	CATGTAGATG	CATAATCAGT

7 i jure 26A

0 02/022	000				
901				AGTACATCAA	
	AGCGATAATG	GTACCACTAC	GCCAAAACCG	TCATGTAGTT	ACCCGCACCT
951				CTCCACCCCA	
	ATCGCCAAAC	TGAGTGCCCC	TAAAGGTTCA	GAGGTGGGGT	AACTGCAGTT
1001				GGACTTTCCA	
	ACCCTCAAAC	AAAACCGTGG	TTTTAGTTGC	CCTGAAAGGT	TTTACAGCAT
1051	ACAACTCCGC	CCCATTGACG	CAAATGGGCG	GTAGGCGTGT	ACGGTGGGAG
	TGTTGAGGCG	GGGTAACTGC	GTTTACCCGC	CATCCGCACA	TGCCACCCTC
1101	GTCTATATAA	GCAGAGCTCG	TTTAGTGAAC	CGTCAGATCG	CCTGGAGACG
				GCAGTCTAGC	
1151	CCATCCACGC	TGTTTTGACC	TCCATAGAAG	ACACCGGGAC	CGATCCAGCC
				TGTGGCCCTG	
1201	TCCGCGGCCG	GGAACGGTGC	ATTGGAACGC	GGATTCCCCG	TGCCAAGAGT
				CCTAAGGGGC	
1251	GAGATCTACC	ATGGCCCCCA	TCTCCCCCAT	TGAGACTGTG	CCTGTGAAGC
				ACTCTGACAC	
1301	TGAAGCCTGG	CATGGATGGC	CCCAAGGTGA	AGCAGTGGCC	CCTGACTGAG
				TCGTCACCGG	
1351	GAGAAGATCA	AGGCCCTGGT	GGAAATCTGC	ACTGAGATGG	AGAAGGAGGG
				TGACTCTACC	
1401	CAAAATCTCC	AAGATTGGCC	CCGAGAACCC	CTACAACACC	CCTGTGTTTG
				GATGTTGTGG	
1451	CCATCAAGAA	GAAGGACTCC	ACCAAGTGGA	GGAAGCTGGT	GGACTTCAGG
,-				CCTTCGACCA	
1501	GAGCTGAACA	AGAGGACCCA	GGACTTCTGG	GAGGTGCAGC	TGGGCATCCC
				CTCCACGTCG	
1551	CCACCCCGCT	GGCCTGAAGA	AGAAGAAGTC	TGTGACTGTG	CTGGCTGTGG
	GGTGGGGCGA	CCGGACTTCT	TCTTCTTCAG	ACACTGACAC	GACCGACACC
1.503	GGGATGCCTA	ammamamamama.	CCCCTCCATC	እርርእሮምምርእር	CAACTACACT
1601				TCCTGAAGTC	
1651	GCCTTCACCA	#***************	СУУСУУДСЯС	ארררריזינור א	TYACGTACCA
1001	CGGAAGTGGT	AGGGGAGGTA	GTTGTTACTC	TGGGGACCGT	AGTCCATGGT
1701	GTACAATGTG	CTGCCCCAGG	GCTGGAAGGG	CTCCCCTGCC	ATCTTCCAGT
2.02				GAGGGGACGG	
1751	CCTCCATGAC	CAAGATYCCTG	GAGCCCTTCA	GGAAGCAGAA	CCCTGACATT
	GGAGGTACTG	GTTCTAGGAC	CTCGGGAAGT	CCTTCGTCTT	GGGACTGTAA
1901	GTGATCTACC	ACTAC ATTGC	TCCCCTCTAT	GTGGGCTCTG	ACCTGGAGAT
1001				CACCCGAGAC	



1901			AAGAAGCACC TTCTTCGTGG		
1951			CCCCGACAAG		
2001			GGACTGTGAA		
	CGACGGACTC	TTCCTGAGGA	CCTGACACTT	ACTGTAGGTC	TTCGACCACC
2051			CAAATCTACC GTTTAGATGG		
2101			CACCAAGGCC GTGGTTCCGG		
2151			AGCTGGCTGA TCGACCGACT		
2201			TATGACCCCT ATACTGGGGA		
2251			CCAGTGGACC GGTCACCTGG		
2301			GCAAGTATGC CGTTCATACG		
2351			ACTGAGGCTG TGACTCCGAC		
2401			GACCCCCAAG CTGGGGGTTC		
2451			GGACTGAGTA CCTGACTCAT		
2501			ACCCCCCCC TGGGGGGGGG		
2551			GGGGGCTGAG CCCCCGACTC		
2601	TGCCAACAGG ACGGTTGTCC	-	TGGGCAAGGC ACCCGTTCCG		
2651	GCAGGCAGAA CGTCCGTCTT		CTGACTGACA GACTGACTGT		
2701	CTCCAGGCCA GAGGTCCGGT		CCTCCAGGAC GGAGGTCCTG		
2751	TGTGACTGCC ACACTGACGG			•	



2851	GAGAAGGTGT	ACCTGGCCTG	GGTGCCTGCC	CACAAGGGCA	TTGGGGGCAA
	CTCTTCCACA	TGGACCGGAC	CCACGGACGG	GTGTTCCCGT	AACCCCCGTT
2901	TGAGCAGGTG	GACAAGCTGG	TGTCTGCTGG	CATCAGGAAG	GTGCTGTTCC
	ACTCGTCCAC	CTGTTCGACC	ACAGACGACC	GTAGTCCTTC	CACGACAAGG
2951	TGGATGGCAT	TGACAAGGCC	CAGGATGAGC	ATGAGAAGTA	CCACTCCAAC
	ACCTACCGTA	ACTGTTCCGG	GTCCTACTCG	TACTCTTCAT	GGTGAGGTTG
3001	TGGAGGGCTA	TGGCCTCTGA	CTTCAACCTG	CCCCTGTGG	TGGCTAAGGA
	ACCTCCCGAT	ACCGGAGACT	GAAGTTGGAC	GGGGGACACC	ACCGATTCCT
3051	GATTGTGGCC	TCCTGTGACA	AGTGCCAGCT	GAAGGGGGAG	GCCATGCATG
	CTAACACCGG	AGGACACTGT	TCACGGTCGA	CTTCCCCCTC	CGGTACGTAC
3101	GGCAGGTGGA	CTGCTCCCCT	GGCATCTGGC	AGCTGGCCTG	CACCCACCTG
	CCGTCCACCT	GACGAGGGGA	CCGTAGACCG	TCGACCGGAC	GTGGGTGGAC
3151	GAGGGCAAGG	TGATCCTGGT	GGCTGTGCAT	GTGGCCTCCG	GCTACATTGA
	CTCCCGTTCC	ACTAGGACCA	CCGACACGTA	CACCGGAGGC	CGATGTAACT
3201	GGCTGAGGTG	ATCCCTGCTG	AGACAGGCCA	GGAGACTGCC	TACTTCCTGC
	CCGACTCCAC	TAGGGACGAC	TCTGTCCGGT	CCTCTGACGG	ATGAAGGACG
3251	TGAAGCTGGC	TGGCAGGTGG	CCTGTGAAGA	CCATCCACAC	TGCCAATGGC
	ACTTCGACCG	ACCGTCCACC	GGACACTTCT	GGTAGGTGTG	ACGGTTACCG
3301	TCCAACTTCA	CTGGGGCCAC	AGTGAGGGCT	GCCTGCTGGT	GGGCTGGCAT
	AGGTTGAAGT	GACCCCGGTG	TCACTCCCGA	CGGACGACCA	CCCGACCGTA
3351	CAAGCAGGAG	TTTGGCATCC	CCTACAACCC	CCAGTCCCAG	GGGGTGGTGG
	GTTCGTCCTC	AAACCGTAGG	GGATGTTGGG	GGTCAGGGTC	CCCCACCACC
3401	CCTCCATGAA	CAAGGAGCTG	AAGAAGATCA	TTGGGCAGGT	GAGGGACCAG
	GGAGGTACTT	GTTCCTCGAC	TTCTTCTAGT	AACCCGTCCA	CTCCCTGGTC
3451	GCTGAGCACC	TGAAGACAGC	TGTGCAGATG	GCTGTGTTCA	TCCACAACTT
	CGACTCGTGG	ACTTCTGTCG	ACACGTCTAC	CGACACAAGT	AGGTGTTGAA
3501	CAAGAGGAAG	GGGGGCATCG	GGGGCTACTC	CGCTGGGGAG	AGGATTGTGG
	GTTCTCCTTC	CCCCCGTAGC	CCCCGATGAG	GCGACCCCTC	TCCTAACACC
3551	ACATCATTGC	CACAGACATC	CAGACCAAGG	AGCTCCAGAA	GCAGATCACC
	TGTAGTAACG	GTGTCTGTAG	GTCTGGTTCC	TCGAGGTCTT	CGTCTAGTGG
3601	AAGATCCAGA	ACTTCAGGGT	GTACTACAGG	GACTCCAGGA	ACCCCCTGTG
	TTCTAGGTCT	TGAAGTCCCA	CATGATGTCC	CTGAGGTCCT	TGGGGGACAC
3651	GAAGGGCCCT	GCCAAGCTGC	TGTGGAAGGG	GGAGGGGGCT	GTGGTGATCC
	CTTCCCGGGA	CGGTTCGACG	ACACCTTCCC	CCTCCCCGA	CACCACTAGG
3701	AGGACAACTC	TGACATCAAG	GTGGTGCCCA	GGAGGAAGGC	CAAGATCATC
	TCCTGTTGAG	ACTGTAGTTC	CACCACGGGT	CCTCCTTCCG	GTTCTAGTAG

7 jure 26 D

3801		TAAAGCCCGG ATTTCGGGCC		
3851		TTGCCCCTCC AACGGGGAGG		
3901.		TCCTTTCCTA AGGAAAGGAT		
3951	•	CATTCTATTC GTAAGATAAG		
4001		GGAAGACAAT CCTTCTGTTA		
4051		GGCGCGCCGT CCGCGCGCCA		
4101		TATAAGGTGG ATATTCCACC		
4151		GCCGCCATGA CGGCGGTACT		
4201		GACAACGCGC CTGTTGCGCG	 	
4251		CCAGCATTGA GGTCGTAACT		
4301		TACGAGACCG ATGCTCTGGC		
4351		TTCAGCCGCT AAGTCGGCGA		
4401		TGAGCCCGCT ACTCGGGCGA		
4451		AAGTTGACGG TTCAACTGCC		
4501		TGTCGTTTCT ACAGCAAAGA		
4551		AGGCTTCCTC TCCGAAGGAG		
4601		TCTGTTTGGA AGACAAACCT		
4651		TTTGCGCGCGC AAACGCGCGC		

Figure 26E

PCT/US01/28861 WO 02/022080

4751	GTTCAGATAC	ATGGGCATAA	GCCCGTCTCT	GGGGTGGAGG	TAGCACCACT
	CAAGTCTATG.	TACCCGTATT	CGGGCAGAGA	CCCCACCTCC	AICGIGGIGA
4801	GCAGAGCTTC	ATGCTGCGGG	GTGGTGTTGT	AGATGATCCA	GTCGTAGCAG
	CGTCTCGAAG	TACGACGCCC	CACCACAACA	TCTACTAGGT	CAGCATCGTC
4851	GAGCGCTGGG	CGTGGTGCCT	AAAAATGTCT	TTCAGTAGCA	AGCTGATTGC
	CTCGCGACCC	GCACCACGGA	TTTTTACAGA	AAGTCATCGT	TCGACTAACG
4901	CAGGGGCAGG	CCCTTGGTGT	AAGTGTTTAC	AAAGCGGTTA	AGCTGGGATG
	GTCCCCGTCC	GGGAACCACA	TTCACAAATG	TTTCGCCAAT	TCGACCCTAC
4951	GGTGCATACG	TGGGGATATG	AGATGCATCT	TGGACTGTAT	TTTTAGGTTG
	CCACGTATGC	ACCCCTATAC	TCTACGTAGA	ACCTGACATA	AAAATCCAAC
5001	CCTATCTTCC	CAGCCATATC	CCTCCGGGGA	TTCATGTTGT	GCAGAACCAC
3001	CGATACAAGG	GTCGGTATAG	GGAGGCCCCT	AAGTACAACA	CGTCTTGGTG
5051	CAGCACAGTG	TATCCGGTGC	ACTTGGGAAA	TTTGTCATGT	AGCTTAGAAG
	GTCGTGTCAC	ATAGGCCACG	TGAACCCTTT	AAACAGTACA	TCGAATCTTC
5101	CAAATCCCTG	GAAGAACTTG	GAGACGCCCT	TGTGACCTCC	AAGATTTTCC
3101	CTTTACGCAC	CTTCTTGAAC	CTCTGCGGGA	ACACTGGAGG	TTCTAAAAGG
					00000000000
5151	ATGCATTCGT	CCATAATGAT	GGCAATGGGC	CCACGGGCGG	CCCCCACCCC
				GGTGCCCGCC	
5201	GAAGATATTT	CTGGGATCAC	TAACGTCATA	GTTGTGTTCC	AGGATGAGAT
	CTTCTATAAA	GACCCTAGTG	ATTGCAGTAT	CAACACAAGG	TCCTACTCTA
5251	CGTCATAGGC	CATTTTTACA	AAGCGCGGGC	GGAGGGTGCC	AGACTGCGGT
3231	GCAGTATCCG	GTAAAAATGT	TTCGCGCCCG	CCTCCCACGG	TCTGACGCCA
5301	ATAATGGTTC	CATCCGGCCC	AGGGGCGTAG	TTACCCTCAC	AGATTTGCAT
	TATTACCAAG	GTAGGCCGGG	TCCCCGCATC	AATGGGAGTG	TCTAAACGIA
5351	TTCCCACGCT	TTGAGTTCAG	ATGGGGGGAT	CATGTCTACC	TGCGGGGCGA
	AAGGGTGCGA	AACTCAAGTC	TACCCCCCTA	GTACAGATGG	ACGCCCCGCT
	5 01101110		CTACCCCACA	телестесса	AGAAAGCAGG
5401	TGAAGAAAAC ACMTCTTTTC	CCAAAGGCCC	CATCCCCTCT	AGTCGACCCT	TCTTTCGTCC
5451	TTCCTGAGCA	GCTGCGACTT	ACCGCAGCCG	GTGGGCCCGT	AAATCACACC
	AAGGACTCGT	CGACGCTGAA	TGGCGTCGGC	CACCCGGGCA	TTTAGTGTGG
5501	TATTACCGGC	TGCAACTGGT	AGTTAAGAGA	GCTGCAGCTG	CCGTCATCCC
5502	ATAATGGCCG	ACGTTGACCA	TCAATTCTCT	CGACGTCGAC	GGCAGTAGGG
5551	TGAGCAGGGG	GGCCACTTCG	TTAAGCATGT	CCCTGACTCG	CATGTTTTCC
	ACTCGTCCCC	CCGGTGAAGC	AATTCGTACA	GGGACTGAGC	GTACAAAAGG
5601	СТСАССАААТ	CCGCCAGAAG	GCGCTCGCCG	CCCAGCGATA	GCAGTTCTTG
	GACTGGTTTA	GGCGGTCTTC	CGCGAGCGGC	GGGTCGCTAT	CGTCAAGAAC

Figure 26 F

5701			AGTTCCAGGC TCAAGGTCCG		
		-			
5751			CAGCATATCT		
	ACGAGATGCC	GTAGAGCTAG	GTCGTATAGA	GGAGCAAAGC	GCCCAACCCC
5801	_		TAGTCGGTGC		
	GCCGAAAGCG	ACATGCCGTC	ATCAGCCACG	AGCAGGTCTG	CCCGGTCCCA
5851	CATGTCTTTC	CACGGGCGCA	GGGTCCTCGT	CAGCGTAGTC	TGGGTCACGG
	GTACAGAAAG	GTGCCCGCGT	CCCAGGAGCA	GTCGCATCAG	ACCCAGTGCC
5901	TGAAGGGGTG	CGCTCCGGGC	TGCGCGCTGG	CCAGGGTGCG	CTTGAGGCTG
	ACTTCCCCAC	GCGAGGCCCG	ACGCGCGACC	GGTCCCACGC	GAACTCCGAC
5951			CTGCCGGTCT		
	CAGGACGACC	ACGACTTCGC	GACGGCCAGA	AGCGGGACGC	GCAGCCGGTC
6001			CATAGTCCAG		
	CATCGTAAAC	TGGTACCACA	GTATCAGGTC	GGGGAGGCGC	CGCACCGGGA
6051	TGGCGCGCAG	CTTGCCCTTG	GAGGAGGCGC	CGCACGAGGG	GCAGTGCAGA
0011			CTCCTCCGCG		
6101	СТТТТСАССС	ССТАСАССТТ	GGGCGCGAGA	AATACCGATT	CCGGGGAGTA
0101			CCCGCGCTCT		
6151	CCCATCCCC	CCGCAGGCCC	CGCAGACGGT	CTCGCATTCC	ACGAGCCAGG
0151			GCGTCTGCCA		
6201			TCAAAAACCA		
	ACTCGAGACC	GGCAAGCCCC	AGTTTTTGGT	CCAAAGGGGG	TACGAAAAAC
6251	ATGCGTTTCT	TACCTCTGGT	TTCCATGAGC	CGGTGTCCAC	GCTCGGTGAC
	TACGCAAAGA	ATGGAGACCA	AAGGTACTCG	GCCACAGGTG	CGAGCCACTG
6301			CGTATACAGA		
•	CTTTTCCGAC	AGGCACAGGG	GCATATGTCT	GAACTCTCCG	GACAGGAGCT
6351			TCGTATAGAA		
	CGCCACAAGG	CGCCAGGAGG	AGCATATCTT	TGAGCCTGGT	GAGACTCTGT
6401	AAGGCTCGCG				
	TTCCGAGCGC	AGGTCCGGTC	GTGCTTCCTC	CGATTCACCC	TCCCCATCGC
6451	GTCGTTGTCC	ACTAGGGGGT	CCACTCGCTC	CAGGGTGTGA	AGACACATGT
	CAGCAACAGG	TGATCCCCCA	GGTGAGCGAG	GTCCCACACT	TCTGTGTACA
6501	CGCCCTCTTC				
	GCGGGAGAAG	CCGTAGTTCC	TTCCACTAAC	CAAACATCCA	CATCCGGTGC
6553	TGACCGGGTG	TTCCTGAAGG	GGGGCTATAA	AAGGGGGTGG	GGGCGCGTTC
			CCCCGATATT		

Figure 266

2/022000	,				
6651	AGTACTCCCT	CTGAAAAGCG	GGCATGACTT	CTGCGCTAAG	ATTGTCAGTT
0031	TCATGAGGGA	GACTTTTCGC	CCGTACTGAA	GACGCGATTC	TAACAGTCAA
6701	TCCAAAAACG	AGGAGGATTT	GATATTCACC	TGGCCCGCGG	TGATGCCTTT
0,02	AGGTTTTTGC	TCCTCCTAAA	CTATAAGTGG	ACCGGGCGCC	ACTACGGAAA
6751	GAGGGTGGCC	GCATCCATCT	GGTCAGAAAA	GACAATCTTT	TTGTTGTCAA
	CTCCCACCGG	CGTAGGTAGA	CCAGTCTTTT	CTGTTAGAAA	AACAACAGTT
6801	GCTTGGTGGC	AAACGACCCG	TAGAGGGCGT	TGGACAGCAA	CTTGGCGATG
	CGAACCACCG	TTTGCTGGGC	ATCTCCCGCA	ACCTGTCGTT	GAACCGCTAC
	•		•		
6851	GAGCGCAGGG	TTTGGTTTTT	GTCGCGATCG	GCGCGCTCCT	TGGCCGCGAT
	CTCGCGTCCC	AAACCAAAAA	CAGCGCTAGC	CGCGCGAGGA	ACCGGCGCTA
			0000110001		CCAAAGACGG
6901	GTTTAGCTGC	ACGTATTCGC	GCGCAACGCA	CCCCCTARCC	CCTTTCTCCC
	CAAATCGACG	TGCATAAGCG	CGCGTTGCGT	GGCGGIANGC	
		amagacca	AGGTGCACGC	CCCAACCGCG	GTTGTGCAGG
6951	TGGTGCGCTC	CACCCCCTCC	TCCACGTGCG	CGGTTGGCGC	CAACACGTCC
	ACCACGLGAG	CAGCCCGIGG	1CCACG10C0		
7001	CTCACAACCT	CAACGCTGGT	GGCTACCTCT	CCGCGTAGGC	GCTCGTTGGT
7001	CACTETTCCA	GTTGCGACCA	CCGATGGAGA	GGCGCATCCG	CGAGCAACCA
Ż051	CCAGCAGAGG	CGGCCGCCCT	TGCGCGAGCA	GAATGGCGGT	AGGGGGTCTA
, , , ,	GGTCGTCTCC	GCCGGCGGGA	ACGCGCTCGT	CTTACCGCCA	TCCCCCAGAT
7101	GCTGCGTCTC	GTCCGGGGGG	TCTGCGTCCA	CGGTAAAGAC	CCCGGGCAGC
	CGACGCAGAG	CAGGCCCCCC	AGACGCAGGT	GCCATTTCTG	GGGCCCGTCG
7151	AGGCGCGCGT	CGAAGTAGTC	TATCTTGCAT	CCTTGCAAGT	CTAGCGCCTG
	TCCGCGCGCA	GCTTCATCAG	ATAGAACGTA	GGAACGTTCA	GATCGCGGAC
			~~~~~~~~~	<b>ごかり かいこうごかい</b>	N CTCCCCCCAC
7201	CTGCCATGCG	CGGGCGGCAA	GCGCGCGCTC CGCGCGCGAG	CATACCCAAC	TCACCCCCTG
	GACGGTACGC	GCCCGCCGTT	CGCGCGCGAG	CHIACCCANC	10
	~~~~~~~~~	CCCCTCCCTC	AGCGCGGAGG	CGTACATGCC	GCAAATGTCG
7251	CCCATGGCAT	CCCCACCCAC	TCGCGCCTCC	GCATGTACGG	CGTTTACAGC
	GGGIACCGIA	CCCCACCCAC			
7301	тааасстаса	GGGGCTCTCT	GAGTATTCCA	AGATATGTAG	GGTAGCATCT
/301	ATTTCCATCT	CCCCGAGAGA	CTCATAAGGT	TCTATACATC	CCATCGTAGA
7351	TCCACCGCGG	ATGCTGGCGC	GCACGTAATC	GTATAGTTCG	TGCGAGGGAG
	AGGTGGCGCC	TACGACCGCG	CGTGCATTAG	CATATCAAGC	ACGCTCCCTC
7401	CGAGGAGGTC	GGGACCGAGG	TTGCTACGGG	CGGGCTGCTC	TGCTCGGAAG
	GCTCCTCCAG	CCCTGGCTCC	AACGATGCCC	GCCCGACGAG	ACGAGCCTTC
				018018180	MATCH CCCTC
7451	ACTATCTGCC	TGAAGATGGC	ATGTGAGTTG	GATGATATGG	TTGGACGCTG
	TGATAGACGG	ACTTCTACCG	TACACTCAAC	CTACTATACC	AACCTGCGAC
			CMCMC10100	መአ ሮሮሮሮሮሞር አ	CCCACCAACC
7501	GAAGACGTTG	AAGCTGGCGT	CACACACACC	TACCOCCTOR	CGCACGAAGG CGCTGCTTCC
	CTTCTGCAAC	TTUGACUGUA	GACACICIGG	HIGGEGENG!	200.32

Figure 26 H

7601					ACTTATCCTG
					TGAATAGGAC
7651					TCGCGGTCTT AGCGCCAGAA
7701	TCCAGTACTC	TTGGATCGGA	AACCCGTCGG	CCTCCGAACG	GTAAGAGCCT
	AGGTCATGAG	AACCTAGCCT	TTGGGCAGCC	GGAGGCTTGC	CATTCTCGGA
7751					CCTTTTCTAC
	TCGTACATCT	TGACCAACTG	CCGGACCATC	CGCGTCGTAG	GGAAAAGATG
7801					TGGGTGAGCG ACCCACTCGC
7851				ACTGGTATTT TGACCATAAA	
7901				AAGTCCGTGC	
	AGCAGCGTAG	GCGGGACGAG	GGTCTCGTTT	TTCAGGCACG	CGAAAAACCT
7951				GTTGAAGAGT	
	TGCGCCTAAA	CCGTCCCGCT	TCCACTGTAG	CAACTTCTCA	TAGAAAGGGC
8001				AGGGTCCCGG	
	GCGCTCCGTA	TTTCAACGCA	CACTACGCCT	TCCCAGGGCC	GTGGAGCCTT
8051	CGGTTGTTAA	TTACCTGGGC	GGCGAGCACG	ATCTCGTCAA	AGCCGTTGAT
	GCCAACAATT	AATGGACCCG	CCGCTCGTGC	TAGAGCAGTT	TCGGCAACTA
8101				GCGCGGGATG	
	CAACACCGGG	TGTTACATTT	CAAGGTTCTT	CGCGCCCTAC	GGGAACTACC
8151				GCTCTTCAGG	
	TTCCGTTAAA	AAATTCAAGG	AGCATCCACT	CGAGAAGTCC	CCTCGACTCG
8201				TGAGGGTTGG	
	GGCACGAGAC	TTTCCCGGGT	CAGACGTTCT	ACTCCCAACC	TTCGCTGCTT
8251	TGAGCTCCAC				
	ACTCGAGGTG	TCCAGTGCCC	GGTAATCGTA	AACGTCCACC	AGCGCTTTCC
8301	TCCTAAACTG				
	AGGATTTGAC	CGCTGGATAC	CGGTAAAAA	GACCCCACTA	CGTCATCTTC
8351	GTAAGCGGGT				
	CATTCGCCCA	GAACAAGGGT	CGCCAGGGTA	GGTTCCAAGC	GCCGATCCAG
8401	TCGCGCGGCA				
	AGCGCGCCGT	CAGTGATCTC	CGAGTAGAGG	CGGCTTGAAG	TACTGGTCGT
8451	TGAAGGGCAC (
	ACTTCCCGTG (CTCGACGAAG	GGTTTCCGGG	GGTAGGTTCA	TATCCAGAGA

Figure 26I

PCT/US01/28861 WO 02/022080

8551	CAACAACTGG	ATCTCCCGCC	ACCAATTGGA	GGAGTGGCTA	TTGATGTGGT
6331		TAGAGGGGGG	TGGTTAACCT	CCTCACCGAT	AACTACACCA
	CTICTIGACC	TAGAGGGGGG			
0601	C C	CTCCCTCCCA	CGGGCCGAAC	ACTCGTGCTG	GCTTTTGTAA
8601	GAAAGTAGAA	CACCCACCCT	CCCCCCTTG	TGAGCACGAC	CGAAAACATT
	CTTCATCTT	CAGGGACGCI	60000110	. 0000	• • • • • • • • • • • • • • • • • • • •
			CCCCTCCACC	GGCTGTACAT	CCTGCACGAG
8651	AAACGTGCGC	AGTACTGGCA	OCCUPATORICA CONTROL	CCGACATGTA	GGACGTGCTC
	TTTGCACGCG	TCATGACCGT	CGCCACGIGC	CCGMCN101N	000010010
			a aa aa.	CACTOCCAAT	יייזיני אַ פַרררריי
8701	GTTGACCTGA	CGACCGCGCA	CAAGGAAGCA	GAGTGGGAAT	AACTCCCCGA
	CAACTGGACT	GCTGGCGCGT	GITCCITCGI	CTCACCCTTA	Anc reduced.
					ጥጥርጥር ርጥጥር እ
8751	CGCCTGGCGG	GTTTGGCTGG	TGGTCTTCTA	CTTCGGCTGC	A A C A C C A A C T
	GCGGACCGCC	CAAACCGACC	ACCAGAAGAT	GAAGCCGACG	AACAGGAAC 1
					20200000
8801	CCGTCTGGCT	GCTCGAGGGG	AGTTACGGTG	GATCGGACCA	CCACGCCGCG
	GGCAGACCGA	CGAGCTCCCC	TCAATGCCAC	CTAGCCTGGT	GGTGCGGCGC
8851	CGAGCCCAAA	GTCCAGATGT	CCGCGCGCGG	CGGTCGGAGC	TTGATGACAA
	GCTCGGGTTT	CAGGTCTACA	GGCGCGCCC	GCCAGCCTCG	AACTACTGTT
8901	CATCGCGCAG	ATGGGAGCTG	TCCATGGTCT	GGAGCTCCCG	CGGCGTCAGG
•••	GTAGCGCGTC	TACCCTCGAC	AGGTACCAGA	CCTCGAGGGC	GCCGCAGTCC
8951	TCAGGCGGGA	GCTCCTGCAG	GTTTACCTCG	CATAGACGGG	TCAGGGCGCG
0,0,0,1	AGTCCGCCCT	CGAGGACGTC	CAAATGGAGC	GTATCTGCCC	AGTCCCGCGC
9001	CCCTAGATCC	AGGTGATACC	TAATTTCCAG	GGGCTGGTTG	GTGGCGGCGT
3001	CCCATCTAGG	TCCACTATGG	ATTAAAGGTC	CCCGACCAAC	CACCGCCGCA
	CCGATCIAGO				
9051	CCATCCCTTC	CAAGAGGCCG	CATCCCCGCG	GCGCGACTAC	GGTACCGCGC
9031	CCTACCCAAC	CTTCTCCGC	GTAGGGGCGC	CGCGCTGATG	CCATGGCGCG
	GCIACCGAAC	GIICICOOO			
01.01		- המכררפרפפפ	GGTGTCCTTG	GATGATGCAT	CTAAAAGCGG
9101	666666666	CCCCCCCCC	CCACAGGAAC	CTACTACGTA	GATTTTCGCC
	CCGCCCGCCA	CCCGGCGCCC	CCACACO.		•
	-0.000000	- CX-CCCCCCC	ACCTACCCC	GGCTCCGGAC	CCGCCGGGAG
9151	TGACGCGGGC	GAGCCCCCGG	TOCATOCOC	CCGAGGCCTG	GCCGCCCTC
	ACTGCGCCCG	CACGGGGGCC	ICCAICCCC		
			רככככככ	GGGCAGGAGC	TGGTGCTGCG
9201	AGGGGGCAGG	GGCACGTCGG	COCCOCOCOC	CCCGTCCTCG	ACCACGACGC
•	TCCCCCGTCC	CCGTGCAGCC	GCGGCGCGCG	CCCGTCCTCG	1100110011000
			0002002000	. CCCCCTTCAT	CTCCTGAATC
9251	CGCGTAGGTT	GCTGGCGAAC	GCGACGACGC	CCCCCAACTA	GAGGACTTAG
	GCGCATCCAA	CGACCGCTTG	CGCTGCTGCG	CCGCCAACIA	GACOMO: 1
			01.0000000	- CMC > CCTTC >	ACCTGAAAGA
9301	TGGCGCCTCI	GCGTGAAGAC	GACGGGCCCG	GIGAGCIIGA	ACCTGAAAGA
	ACCGCGGAGA	CGCACTTCTG	CIGCCCGGGC	. CMCTCGAMCT	TGGACTTTCT
					TO CO CO CO A A A A
9351	GAGTTCGACA	GAATCAATTI	CGGTGTCGTT	COCCOCCOCC	TGGCGCAAAA
	CTCAAGCTGT	CTTAGTTAAA	GCCACAGCA	CTGCCGCCGG	ACCGCGTTTT
					, ,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,
9401	TCTCCTGCAC	GTCTCCTGAG	TTGTCTTGAT	AGGCGATCTC	GGCCATGAAC
	AGAGGACGT	CAGAGGACTC	AACAGAACTA	A TCCGCTAGAG	CCGGTACTTG

Figure 26 J

9501	GGCGGCGAGG CCGCCGCTCC				AAGGCGTTGA TTCCGCAACT
9551					TTCGGCATCG AAGCCGTAGC
9601			-		GCCGGGCGAA CGGCCCGCTT
9651					GTGGTGGCGG CACCACCGCC
9701				AGCGTCGCAA TCGCAGCGTT	CGTGGATTCG GCACCTAAGC
9 751				ATGGCCTCGT TACCGGAGCA	
9801				CGACACGGTT GCTGTGCCAA	
9851				CGCGCACCTC GCGCGTGGAG	
9901				TCCTCTTCCA AGGAGAAGGT	
9951				AGGGGGGACA TCCCCCTGT	
10001				GCTCGATCAT CGAGCTAGTA	
10051				CCGTTCTCGC GGCAAGAGCG	
10101				ATGGGTTGGC TACCCAACCG	
10151		-		ATCTCAACAA TAGAGTTGTT	
10201	GGTACTCCGC CCATGAGGCG			TCCGCATCGA AGGCGTAGCT	-
10251	AAACCTCTCG TTTGGAGAGC			ACAGTCGCAA TGTCAGCGTT	
10301	GCACCGTGGC CGTGGCACCG				
10351	GTGCTGCTGA CACGACGACT			GTCTTGAGAC CAGAACTCTG	

Figure 26 K

10451	CGGCCATGCC	CCAGGCTTCG	TTTTGACATC	GGCGCAGGTC	TTTGTAGTAG
	GCCGGTACGG	GGTCCGAAGC	AAAACTGTAG	CCGCGTCCAG	AAACATCATC
10501	TCTTGCATGA	GCCTTTCTAC	CGGCACTTCT	TCTTCTCCTT	CCTCTTGTCC
	AGAACGTACT	CGGAAAGATG	GCCGTGAAGA	AGAAGAGGAA	GGAGAACAGG
10551	TGCATCTCTT	GCATCTATCG	CTGCGGCGGC	GGCGGAGTTT	GGCCGTAGGT
	ACGTAGAGAA	CGTAGATAGC	GACGCCGCCG	CCGCCTCAAA	CCGGCATCCA
10601	GGCGCCCTCT	TCCTCCCATG	CGTGTGACCC	CGAAGCCCCT	CATCGGCTGA
	CCGCGGGAGA	AGGAGGGTAC	GCACACTGGG	GCTTCGGGGA	GTAGCCGACT
10651	AGCAGGGCTA	GGTCGGCGAC	AACGCGCTCG	GCTAATATGG	CCTGCTGCAC
	TCGTCCCGAT	CCAGCCGCTG	TTGCGCGAGC	CGATTATACC	GGACGACGTG
10701	CTGCGTGAGG	GTAGACTGGA	AGTCATCCAT	GTCCACAAAG	CGGTGGTATG
	GACGCACTCC	CATCTGACCT	TCAGTAGGTA	CAGGTGTTTC	GCCACCATAC
10751	CGCCCGTGTT	GATGGTGTAA	GTGCAGTTGG	CCATAACGGA	CCAGTTAACG
	GCGGGCACAA	CTACCACATT	CACGTCAACC	GGTATTGCCT	GGTCAATTGC
10801	GTCTGGTGAC	CCGGCTGCGA	GAGCTCGGTG	TACCTGAGAC	GCGAGTAAGC
	CAGACCACTG	GGCCGACGCT	CTCGAGCCAC	ATGGACTCTG	CGCTCATTCG
10851	GGAGCTCAGT	TTATGCATCA	GCAACGTTCA	CCGCACCAGG GGCGTGGTCC	ATGACCATAG
10901	GGTGGTTTTT	CACGCCGCCG	CCGACCGCCA	AGAGGGGCCA TCTCCCCGGT	CGCATCCCAC
10951	GCCGGGGCTC	CGGGGGCGAG	ATCTTCCAAC	ATAAGGCGAT	GATATCCGTA
	CGGCCCCGAG	GCCCCCGCTC	TAGAAGGTTG	TATTCCGCTA	CTATAGGCAT
11001	CTACATGGAC	CTGTAGGTCC	ACTACGGCCG	GGCGGTGGTG CCGCCACCAC	CTCCGCGCGC
11051	CTTTCAGCGC	CTGCGCCAAG	GTCTACAACG	GCAGCGGCAA CGTCGCCGTT	TTTCACGAGG
11101	TACCAGCCCT	GCGAGACCGG	CCAGTCCGCG	GCGCAATCGT CGCGTTAGCA	ACTGCGAGAT
	CTGGCACGTT	TTCCTCTCGG	ACATTCGCCC	GTGAGAAGGC	TGGTCTGGTG ACCAGACCAC
	CTATTTAAGC	GTTCCCATAG	TACCGCCTGC	TGGCCCCAAG	GAGCCCCGTA CTCGGGGCAT
	AGGCCGGCAG	GCGGCACTAG	GTACGCCAAT	GGCGGGCGCA	GTCGAACCCA CAGCTTGGGT
11301	GGTGTGCGAC	GTCAGACAAC	GGGGGAGTGC	TCCTTTTGGC	TTCCTTCCAG
	CCACACGCTG	CAGTCTGTTG	CCCCTCACG	AGGAAAACCG	AAGGAAGGTC

7igure 26L

11401				AGTGGCTCGC TCACCGAGCG	
11451				GGGACCCCCG CCCTGGGGGC	
11501				TTGCCTCCCC AACGGAGGGG	
11551				GACGAGCCCC CTGCTCGGGG	
11601				GCGCCCCCT CGCGGGGGGA	
11651				GGGCACCCTC CCCGTGGGAG	
11701				GACGCGGCAG CTGCGCCGTC	
11751				CTACCTGGAC GATGGACCTG	
11801				CTCCTGAGCG GAGGACTCGC	
11851				TACGTGCCGC ATGCACGGCG	
11901				GGAGATGCGG CCTCTACGCC	
11951				TGAATCGCGA ACTTAGCGCT	
12001				ACCGGGATTA TGGCCCTAAT	
12051				CGCATACGAG GCGTATGCTC	
12101	ACCAGGAGAT TGGTCCTCTA			ACAACCACGT TGTTGGTGCA	
12151	GTGGCGCGCG CACCGCGCGC			ATGCATCTGT TACGTAGACA	
12201	AAGCGCGCTG TTCGCGCGAC				
12251	TCCTTATAGT AGGAATATCA	•			
			4		

7 igure 26 M

12351	CCTGCAGAGC	ATAGTGGTGC	AGGAGCGCAG	CTTGAGCCTG	GCTGACAAGG
12331	GGACGTCTCG	TATCACCACG	TCCTCGCGTC	GAACTCGGAC	CGACTGTTCC
12401	TECCCECCAT	CAACTATTCC	ATGCTTAGCC	TGGGCAAGTT	TTACGCCCGC
12401	ACCGGCGGTA	GTTGATAAGG	TACGAATCGG	ACCCGTTCAA	AATGCGGGCG
12451	AAGATATACC	ATACCCCTTA	CGTTCCCATA	GACAAGGAGG	TAAAGATCGA
				CTGTTCCTCC	
12501	GGGGTTCTAC	ATGCGCATGG	CGCTGAAGGT	GCTTACCTTG	AGCGACGACC
				CGAATGGAAC	
12551	TGGGCGTTTA	TCGCAACGAG	CGCATCCACA	AGGCCGTGAG	CGTGAGCCGG
				TCCGGCACTC	
12601	CGGCGCGAGC	TCAGCGACCG	CGAGCTGATG	CACAGCCTGC	AAAGGGCCCT
				GTGTCGGACG	
12651	GGCTGGCACG	GGCAGCGGCG	ATAGAGAGGC	CGAGTCCTAC	TTTGACGCGG
,				GCTCAGGATG	
12701	GCGCTGACCT	GCGCTGGGCC	CCAAGCCGAC	GCGCCCTGGA	GGCAGCTGGG
	*			CGCGGGACCT	
12751	GCCGGACCTG	GGCTGGCGGT	GGCACCCGCG	CGCGCTGGCA	ACGTCGGCGG
				GCGCGACCGT	
12801	CGTGGAGGAA	TATGACGAGG	ACGATGAGTA	CGAGCCAGAG	GACGGCGAGT
					CTGCCGCTCA
12851	ACTAAGCGGT	GATGTTTCTG	ATCAGATGAT	GCAAGACGCA	ACGGACCCGG
					TGCCTGGGCC
12901	CGGTGCGGGC	GGCGCTGCAG	AGCCAGCCGT	CCGGCCTTAA	CTCCACGGAC
					GAGGTGCCTG
12951	GACTGGCGCC	AGGTCATGGA	CCGCATCATG	TCGCTGACTG	CGCGCAATCC
					GCGCGTTAGG
13001	TGACGCGTTC	CGGCAGCAGC	CGCAGGCCAA	CCGGCTCTCC	GCAATTCTGG
					CGTTAAGACC
13051	AAGCGGTGGT	CCCGCCGC	GCAAACCCCA	CGCACGAGAA	GCTGCTGGCG
					CCACGACCGC
13101	ATCGTAAACG	CGCTGGCCGA	AAACAGGGCC	ATCCGGCCCG	ACGAGGCCGG
					TGCTCCGGCC
13151	CCTGGTCTAC	GACGCGCTGC	TTCAGCGCGT	GGCTCGTTAC	AACAGCGGCA
	-				TTGTCGCCGT
13201	ACGTGCAGAC	CAACCTGGAC	CGGCTGGTGG	GGGATGTGCG	CGAGGCCGTG
	TGCACGTCTG	GTTGGACCTG	GCCGACCACC	CCCTACACGC	GCTCCGGCAC

Figure 26 N

13301		TTCCTGAGTA AAGGACTCAT		
13351		CAACTTTGTG GTTGAAACAC		
13401		AGGTGTACCA TCCACATGGT		
13451		CTGCAGACCG GACGTCTGGC		
13501		GGGGGTGCGG CCCCCACGCC		
13551		CGCCCAACTC GCGGGTTGAG		
13601		GGCAGCGTGT CCGTCGCACA		
13651		CGAGGCCATA GCTCCGGTAT		
13701		CAAGTGTCAG GTTCACAGTC		
13751		ACCCTAAACT TGGGATTTGA		
13801		CAGTTTAAAC GTCAAATTTG		
13851		TGAGCCTTAA ACTCGGAATT		
13901		ATGACCGCGC TACTGGCGCG		
13951		TATCAACCGC ATAGTTGGCG		
14001	GTGAACCCCG CACTTGGGGC	AGTATTTCAC TCATAAAGTG		
14051	GCCCCCTGGT CGGGGGACCA	TTCTACACCG AAGATGTGGC		
14101	GATTCCTCTG CTAAGGAGAC	GGACGACATA CCTGCTGTAT	 	
14151	ACCCTGCTAG TGGGACGATC	AGTTGCAACA TCAACGTTGT	•	

7, gure 260

14251	CGCGGTCAGA	TGCTAGTAGC	CCATTTCCAA	GCTTGATAGG	GTCTCTTACC
	GCGCCAGTCT	ACGATCATCG	GGTAAAGGTT	CGAACTATCC	CAGAGAATGG
14301	AGCACTCGCA	CCACCGGCC	GCGCCTGCTG	GGCGAGGAGG	AGTACCTAAA
	TCGTGAGCGT	GGTGGGCGGG	CGCGGACGAC	CCGCTCCTCC	TCATGGATTT
14351	CAACTCGCTG	CTGCAGCCGC	AGCGCGAAAA	AAACCTGCCT	CCGGCATTTC
	GTTGAGCGAC	GACGTCGGCG	TCGCGCTTTT	TTTGGACGGA	GGCCGTAAAG
14401	CCAACAACGG	GATAGAGAGC	CTAGTGGACA	AGATGAGTAG	ATGGAAGACG
	GGTTGTTGCC	CTATCTCTCG	GATCACCTGT	TCTACTCATC	TACCTTCTGC
14451	TACGCGCAGG ATGCGCGTCC	AGCACAGGGA TCGTGTCCCT	CGTGCCAGGC GCACGGTCCG	GCGCGCCGC	CCACCCGTCG GGTGGGCAGC
14501	TCAAAGGCAC	GACCGTCAGC	GGGGTCTGGT	GTGGGAGGAC	GATGACTCGG
	AGTTTCCGTG	CTGGCAGTCG	CCCCAGACCA	CACCCTCCTG	CTACTGAGCC
14551	CAGACGACAG	CAGCGTCCTG	GATTTGGGAG	GGAGTGGCAA	CCCGTTTGCG
	GTCTGCTGTC	GTCGCAGGAC	CTAAACCCTC	CCTCACCGTT	GGGCAAACGC
14601	CACCTTCGCC	CCAGGCTGGG	GAGAATGTTT	TAAAAAAAAA	AAAAGCATGA
	GTGGAAGCGG	GGTCCGACCC	CTCTTACAAA	ATTTTTTTTT	TTTTCGTACT
14651	TGCAAAATAA	AAAACTCACC	AAGGCCATGG	CACCGAGCGT	TGGTTTTCTT
	ACGTTTTATT	TTTTGAGTGG	TTCCGGTACC	GTGGCTCGCA	ACCAAAAGAA
14701	GTATTCCCCT	TAGTATGCGG	CGCGCGGCGA	TGTATGAGGA	AGGTCCTCCT
	CATAAGGGGA	ATCATACGCC	GCGCGCCGCT	ACATACTCCT	TCCAGGAGGA
14751	CCCTCCTACG	AGAGTGTGGT	GAGCGCGGCG	CCAGTGGCGG	CGGCGCTGGG
	GGGAGGATGC	TCTCACACCA	CTCGCGCCGC	GGTCACCGCC	GCCGCGACCC
14801	TTCTCCCTTC AAGAGGGAAG	GATGCTCCCC CTACGAGGGG	TGGACCCGCC ACCTGGGCGG	GTTTGTGCCT CAAACACĢGA	CCGCGGTACC
14851	TGCGGCCTAC	CGGGGGGAGA	AACAGCATCC	GTTACTCTGA	GTTGGCACCC
	ACGCCGGATG	GCCCCCCTCT	TTGTCGTAGG	CAATGAGACT	CAACCGTGGG
14901	CTATTCGACA	CCACCCGTGT	GTACCTGGTG	GACAACAAGT	CAACGGATGT
	GATAAGCTGT	GGTGGGCACA	CATGGACCAC	CTGTTGTTCA	GTTGCCTACA
14951	GGCATCCCTG	AACTACCAGA	ACGACCACAG	CAACTTTCTG	ACCACGGTCA
	CCGTAGGGAC	TTGATGGTCT	TGCTGGTGTC	GTTGAAAGAC	TGGTGCCAGT
15001	TTCAAAACAA AAGTTTTGTT	TGACTACAGC ACTGATGTCG	CCGGGGGAGG	CAAGCACACA GTTCGTGTGT	GACCATCAAT CTGGTAGTTA
15051	CTTGACGACC	GGTCGCACTG	GGGCGGCGAC	CTGAAAACCA	TCCTGCATAC
	GAACTGCTGG	CCAGCGTGAC	CCCGCCGCTG	GACTTTTGGT	AGGACGTATG
15101	CAACATGCCA	AATGTGAACG	AGTTCATGTT	TACCAATAAG	TTTAAGGCGC
	GTTGTACGGT	TTACACTTGC	TCAAGTACAA	ATGGTTATTC	AAATTCCGCG

Figure 26 P

WO 02/022080 PCT/US01/28861 15151 GGGTGATGGT GTCGCGCTTG CCTACTAAGG ACAATCAGGT GGAGCTGAAA CCCACTACCA CAGCGCGAAC GGATGATTCC TGTTAGTCCA CCTCGACTTT 15201 TACGAGTGGG TGGAGTTCAC GCTGCCCGAG GGCAACTACT CCGAGACCAT ATGCTCACCC ACCTCAAGTG CGACGGGCTC CCGTTGATGA GGCTCTGGTA 15251 GACCATAGAC CTTATGAACA ACGCGATCGT GGAGCACTAC TTGAAAGTGG CTGGTATCTG GAATACTTGT TGCGCTAGCA CCTCGTGATG AACTTTCACC 15301 GCAGACAGAA CGGGGTTCTG GAAAGCGACA TCGGGGTAAA GTTTGACACC CGTCTGTCTT GCCCCAAGAC CTTTCGCTGT AGCCCCATTT CAAACTGTGG 15351 CGCAACTTCA GACTGGGGTT TGACCCCGTC ACTGGTCTTG TCATGCCTGG GCGTTGAAGT CTGACCCCAA ACTGGGGCAG TGACCAGAAC AGTACGGACC 15401 GGTATATACA AACGAAGCCT TCCATCCAGA CATCATTTTG CTGCCAGGAT CCATATATGT TTGCTTCGGA AGGTAGGTCT GTAGTAAAAC GACGGTCCTA 15451 GCGGGGTGGA CTTCACCCAC AGCCGCCTGA GCAACTTGTT GGGCATCCGC CGCCCACCT GAAGTGGGTG TCGGCGGACT CGTTGAACAA CCCGTAGGCG 15501 AAGCGGCAAC CCTTCCAGGA GGGCTTTAGG ATCACCTACG ATGATCTGGA TTCGCCGTTG GGAAGGTCCT CCCGAAATCC TAGTGGATGC TACTAGACCT 15551 GGGTGGTAAC ATTCCCGCAC TGTTGGATGT GGACGCCTAC CAGGCGAGCT CCCACCATTG TAAGGGCGTG ACAACCTACA CCTGCGGATG GTCCGCTCGA 15601 TGAAAGATGA CACCGAACAG GGCGGGGGTG GCGCAGGCGG CAGCAACAGC ACTITICIACT GTGGCTTGTC CCGCCCCCAC CGCGTCCGCC GTCGTTGTCG 15651 AGTGGCAGCG GCGCGGAAGA GAACTCCAAC GCGGCAGCCG CGGCAATGCA TCACCGTCGC CGCGCCTTCT CTTGAGGTTG CGCCGTCGGC GCCGTTACGT 15701 GCCGGTGGAG GACATGAACG ATCATGCCAT TCGCGGCGAC ACCTTTGCCA CGGCCACCTC CTGTACTTGC TAGTACGGTA AGCGCCGCTG TGGAAACGGT 15751 CACGGGCTGA GGAGAAGCGC GCTGAGGCCG AAGCAGCGGC CGAAGCTGCC GTGCCCGACT CCTCTTCGCG CGACTCCGGC TTCGTCGCCG GCTTCGACGG 15801 GCCCCGCTG CGCAACCCGA GGTCGAGAAG CCTCAGAAGA AACCGGTGAT CGGGGGCGAC GCGTTGGGCT CCAGCTCTTC GGAGTCTTCT TTGGCCACTA 15851 CANACCCCTG ACAGAGGACA GCAAGAAACG CAGTTACAAC CTAATAAGCA GTTTGGGGAC TGTCTCCTGT CGTTCTTTGC GTCAATGTTG GATTATTCGT 15901 ATGACAGCAC CTTCACCCAG TACCGCAGCT GGTACCTTGC ATACAACTAC TACTGTCGTG GAAGTGGGTC ATGCCGTCGA CCATGGAACG TATGTTGATG 15951 GGCGACCCTC AGACCGGAAT CCGCTCATGG ACCCTGCTTT GCACTCCTGA CCGCTGGGAG TCTGGCCTTA GGCGAGTACC TGGGACGAAA CGTGAGGACT 16001 CGTAACCTGC GGCTCGGAGC AGGTCTACTG GTCGTTGCCA GACATGATGC GCATTGGACG CCGAGCCTCG TCCAGATGAC CAGCAACGGT CTGTACTACG 16051 AAGACCCCGT GACCTTCCGC TCCACGCGCC AGATCAGCAA CTTTCCGGTG

Figure 26 Q

TTCTGGGGCA CTGGAAGGCG AGGTGCGCGG TCTAGTCGTT GAAAGGCCAC

16151	GGCCGTCTAC	TCCCAACTCA	TCCGCCAGTT	TACCTCTCTG	ACCCACGTGT
	CCGGCAGATG	AGGGTTGAGT	AGGCGGTCAA	ATGGAGAGAC	TGGGTGCACA
16201	TCAATCGCTT AGTTAGCGAA	TCCCGAGAAC AGGGCTCTTG	CAGATTTTGG GTCTAAAACC	GCGCGCGCC	AGCCCCCACC TCGGGGGTGG
16251	ATCACCACCG	TCAGTGAAAA	CGTTCCTGCT	CTCACAGATC	ACGGGACGCT
	TAGTGGTGGC	AGTCACTTTT	GCAAGGACGA	GAGTGTCTAG	TGCCCTGCGA
16301	ACCGCTGCGC	AACAGCATCG	GAGGAGTCCA	GCGAGTGACC	ATTACTGACG
	TGGCGACGCG	TTGTCGTAGC	CTCCTCAGGT	CGCTCACTGG	TAATGACTGC
16351	GGTCTGCGGC	GTGGACGGGG	TACGTTTACA ATGCAAATGT	TCCGGGACCC	GTATCAGAGC
16401	CCGCGCGTCC	TATCGAGCCG	CACTTTTTGA	GCAAGCATGT	CCATCCTTAT
	GGCGCGCAGG	ATAGCTCGGC	GTGAAAAACT	CGTTCGTACA	GGTAGGAATA
16451	ATCGCCCAGC	AATAACACAG	GCTGGGGCCT	GCGCTTCCCA	AGCAAGATGT
	TAGCGGGTCG	TTATTGTGTC	CGACCCCGGA	CGCGAAGGGT	TCGTTCTACA
16501	TTGGCGGGGC	CAAGAAGCGC	TCCGACCAAC	ACCCAGTGCG	CGTGCGCGGG
	AACCGCCCCG	GTTCTTCGCG	AGGCTGGTTG	TGGGTCACGC	GCACGCGCCC
16551	CACTACCGCG GTGATGGCGC	CGCCCTGGGG GCGGGACCCC	CGCGCACAAA GCGCGTGTTT	CGCGGCCGCA	CTGGGCGCAC GACCCGCGTG
16601	CACCGTCGAT	GACGCCATCG	ACGCGGTGGT	GGAGGAGGCG	CGCAACTACA
	GTGGCAGCTA	CTGCGGTAGC	TGCGCCACCA	CCTCCTCCGC	GCGTTGATGT
16651	CGCCCACGCC	GCCACCAGTG	TCCACAGTGG	ACGCGGCCAT	TCAGACCGTG
	GCGGGTGCGG	CGGTGGTCAC	AGGTGTCACC	TGCGCCGGTA	AGTCTGGCAC
16701	GTGCGCGGAG	CCCGGCGCTA	TGCTAAAATG	AAGAGACGGC	GGAGGCGCGT
	CACGCGCCTC	GGGCCGCGAT	ACGATTTTAC	TTCTCTGCCG	CCTCCGCGCA
16751	AGCACGTCGC TCGTGCAGCG	CACCGCCGCC GTGGCGGCGG	GACCCGGCAC CTGGGCCGTG	TGCCGCCCAA ACGGCGGGTT	CGCGCGCCCC
16801	CGGCCCTGCT GCCGGGACGA	TAACCGCGCA ATTGGCGCGT	CGTCGCACCG GCAGCGTGGC	GCCGACGGGC CGGCTGCCCG	GGCCATGCGG
16851	GCCGCTCGAA	GGCTGGCCGC	GGGTATTGTC	ACTGTGCCCC	CCAGGTCCAG
	CGGCGAGCTT	CCGACCGGCG	CCCATAACAG	TGACACGGGG	GGTCCAGGTC
16901	GCGACGAGCG CGCTGCTCGC	GCCGCCGCAG CGGCGGCGTC	CAGCCGCGCCGCGCGCGCGGCGCGGCGGCGGCGGGCGGG	CATTAGTGCT GTAATCACGA	ATGACTCAGG TACTGAGTCC
16951	GTCGCAGGGG CAGCGTCCCC	CAACGTGTAT GTTGCACATA	TGGGTGCGCG	ACTCGGTTAG TGAGCCAATC	CGGCCTGCGC GCCGGACGCG
17001	GTGCCCGTGC CACGGGCACG	GCACCCGCCC	CCCGCGCAAC	TAGATTGCAA ATCTAACGTT	GAAAAAACTA CTTTTTTGAT

7igure 26R

17101		GCGCAAAATC CGCGTTTTAG			CATCGCGCCG
17151					AGCCCCGAAA
1,131		CGGGGGGCTT		-	
17201		GTCAAAAAGA CAGTTTTTCT			
17251		ACTGCTGCAC TGACGACGTG			
17301		GCGTAAAACG CGCATTTTGC			
17351		GAGCGCTCCA CTCGCGAGGT			
17401	-	CGAGGACCTG GCTCCTGGAC			
17451	<u> ጥጥጥ</u> ርርር ጥ አርር	GAAAGCGGCA	TARCCACATC		CCCTCCACCA
1/401		CTTTCGCCGT			
17501	GGGCAACCCA	ACACCTAGCC	TAAAGCCCCT	AACACTGCAG	CAGGTGCTGC
1,301		TGTGGATCGG			
17551	CCGCGCTTGC	ACCGTCCGAA	GAAAAGCGCG	GCCTAAAGCG	CGAGTCTGGT
		TGGCAGGCTT			
17601	GACTTGGCAC	CCACCGTGCA	GCTGATGGTA	CCCAAGCGCC	AGCGACTGGA
		GGTGGCACGT			
17651	AGATGTCTTG	GAAAAAATGA	CCGTGGAACC	TGGGCTGGAG	CCCGAGGTCC
	TCTACAGAAC	CTTTTTTACT	GGCACCTTGG	ACCCGACCTC	GGGCTCCAGG
17701	GCGTGCGGCC	AATCAAGCAG	GTGGCGCCGG	GACTGGGCGT	GCAGACCGTG
		TTAGTTCGTC			
17751	GACGTTCAGA CTGCAAGTCT	TACCCACTAC ATGGGTGATG	CAGTAGCACC GTCATCGTGG	AGTATTGCCA TCATAACGGT	CCGCCACAGA GGCGGTGTCT
	0000100010	-01C1110CM	00000000000	000 0000000	000018000
17801	GGGCATGGAG CCCGTACCTC	TGTGTTTGCA			
17851	CGGTGCAGGC	CCTCCCTCCC	CCCCCCTCCA	אכארריתרידאר	GGAGGTGCAA
¥103I	••	CCAGCGACGC			
17901	ACGGACCCGT TGCCTGGGCA	GGATGTTTCG CCTACAAAGC			
17951	GAGGAAGTAC	GGCGCCCCCA	GCGCGCTACT	GCCCGAATAT	GCCCTACATC
±1231		CCGCGGCGGT			

Figure 265

18051	AGACGAGCAA TCTGCTCGTT	CTACCCGACG GATGGGCTGC	CCGAACCACC GGCTTGGTGG	ACTGGAACCC TGACCTTGGG	CGGCGGCGGC
18101	TCGCCGTCGC	CAGCCCGTGC	TGGCCCCGAT	TTCCGTGCGC	AGGGTGGCTC
	AGCGGCAGCG	GTCGGGCACG	ACCGGGGCTA	AAGGCACGCG	TCCCACCGAG
18151	GCGAAGGAGG	CAGGACCCTG	GTGCTGCCAA	CAGCGCGCTA	CCACCCCAGC
	CGCTTCCTCC	GTCCTGGGAC	CACGACGGTT	GTCGCGCGAT	GGTGGGGTCG
18201	ATCGTTTAAA	AGCCGGTCTT	TGTGGTTCTT	GCAGATATGG	CCCTCACCTG
	TAGCAAATTT	TCGGCCAGAA	ACACCAAGAA	CGTCTATACC	GGGAGTGGAC
18251	GGCGGAGGCA	AAGGGCCACG	CGGGATTCCG GCCCTAAGGC	TCCTTCTTAC	GTGGCATCCT
18301	CCCCGTACCG	GCCGGTGCCG	CTGACGGGCG GACTGCCCGC	CGTACGCAGC	ACGCGTGGTG
18351	GCCGCCGCCG	CGCGCAGCGT	CCGTCGCATG GGCAGCGTAC	GCGCCGCCAT	AGGACGGGGA
18401	GGAATAAGGT	GACTAGCGGC	CGGCGATTGG GCCGCTAACC	GCGGCACGGG	CCTTAACGTA
18451	CCGTGGCCTT	GCAGGCGCAG	AGACACTGAT	TAAAAACAAG	TTGCATGTGG
	GGCACCGGAA	CGTCCGCGTC	TCTGTGACTA	ATTTTTGTTC	AACGTACACC
18501	AAAAATCAAA	ATAAAAAGTC	TGGACTCTCA	CGCTCGCTTG	GTCCTGTAAC
	TTTTTAGTTT	TATTTTTCAG	ACCTGAGAGT	GCGAGCGAAC	CAGGACATTG
18551	TATT ITGTAG	AATGGAAGAC	ATCAACTTTG	CGTCTCTGGC	CCCGCGACAC
	ATAAAACATC	TTACCTTCTG	TAGTTGAAAC	GCAGAGACCG	GGGCGCTGTG
18601	GGCTCGCGCC	CGTTCATGGG	AAACTGGCAA	GATATCGGCA	CCAGCAATAT
	CCGAGCGCGG	GCAAGTACCC	TTTGACCGTT	CTATAGCCGT	GGTCGTTATA
18651	GAGCGGTGGC	GCCTTCAGCT	GGGGCTCGCT	GTGGAGCGGC	TTAAAAATT
	CTCGCCACCG	CGGAAGTCGA	CCCCGAGCGA	CACCTCGCCG	TAATTTTAA
18701	TCGGTTCCAC	CGTTAAGAAC	TATGGCAGCA	AGGCCTGGAA	CAGCAGCACA
	AGCCAAGGTG	GCAATTCTTG	ATACCGTCGT	TCCGGACCTT	GTCGTCGTGT
18751	GGCCAGATGC	TGAGGGATAA	GTTGAAAGAG	CAAAATTTCC	AACAAAAGGT
	CCGGTCTACG	ACTCCCTATT	CAACTTTCTC	GTTTTAAAGG	TTGTTTTCCA
18801	GGTAGATGGC	CTGGCCTCTG	GCATTAGCGG	GGTGGTGGAC	CTGGCCAACC
	CCATCTACCG	GACCGGAGAC	CGTAATCGCC	CCACCACCTG	GACCGGTTGG
18851	AGGCAGTGCA TCCGTCACGT	AAATAAGATT TTTATTCTAA	AACAGTAAGC TTGTCATTCG	TTGATCCCCG AACTAGGGGC	CCCTCCCGTA
18901	GAGGAGCCTC	CACCGGCCGT	GGAGACAGTG	TCTCCAGAGG	GGCGTGGCGA
	CTCCTCGGAG	GTGGCCGGCA	CCTCTGTCAC	AGAGGTCTCC	CCGCACCGCT

Figure 26T

19001				CACCACCCGT GTGGTGGGCA
19051		CCATGGCTAC GGTACCGATG	 	- · · · ·
19101	GCTGGACCTG CGACCTGGAC	CCTCCCCCG GGAGGGGGGC		
19151		CGTTGTTGTA GCAACAACAT	 	
19201		GTCCGCGATC CAGGCGCTAG		
19251		AACAGCATCG TTGTCGTAGC		
19301		CTGATAGCTA GACTATCGAT		
19351		CAGAGGAGCT GTCTCCTCGA		
19401		TTCGATGATG AAGCTACTAC	 	
19451		CGGAGTACCT GCCTCATGGA		
19501	•	TACTTCAGCC ATGAAGTCGG	 	
19551		CGACGTGACC GCTGCACTGG		
19601		TGGACCGTGA ACCTGGCACT	 	
19651				TCCACGTACT AGGTGCATGA
19701				GCCCTACTCT CGGGATGAGA
19751		ACAACGCCCT TGTTGCGGGA		ATCCTTGCGA TAGGAACGCT
19801		GCTGCTACTG CGACGATGAC		GAAGAGGACG CTTCTCCTGC
19851	ATGACAACGA TACTGTTGCT	AGACGAAGTA TCTGCTTCAT		

Figure 26 U

19951	TCAAATAGGT	GTCGAAGGTC	AAACACCTAA	ATATGCCGAT	AAAACATTTC
	ACTITATICA	CAGCTTCCAG	TTTGTGGATT	TATACGGCTA	TTTTGTAAAG
	A01111110011				
00001	**********	TCAAATAGGA	CAATCTCAGT	CCTACGAAAC	AGAAATTAAT
20001	AACCIGAACC	AGTTTATCCT	COMPACACACA	CCATCCTTTC	ፈሊታፈ ጀታታታ ማ
	TTGGACTTGG	AGTITATECT	CTTAGAGTCA	CCAIGCIIIG	ICIIIAAIAA
					* * * * * * * * * * * * * * * * * * *
20051	CATGCAGCTG	GGAGAGTCCT	AAAAAAGACT	ACCCCAATGA	AACCAIGIIA
	GTACGTCGAC	CCTCTCAGGA	TTTTTTCTGA	TGGGGTTACT	TTGGTACAAT
20101	CGGTTCATAT	GCAAAACCCA	CAAATGAAAA	TGGAGGGCAA	GGCATTCTTG
	GCCAAGTATA	CGTTTTGGGT	GTTTACTTTT	ACCTCCCGTT	CCGTAAGAAC
		•			
20151	TABACCAACA	AAATGGAAAG	CTAGAAAGTC	AAGTGGAAAT	GCAATTTTTC
20131	* DOUBLE COURTE	TTTACCTTTC	CATCTTTCAG	TTCACCTTTA	CGTTAAAAAG
•	ATTICGITGI	IIIACCIIIC	GATCTICAG		•
		AGGCAGCCGC	A CCC A A TCCT	СУДУУСТТСУ	CTCCTAAAGT
20201	TCAACTACTG	TCCGTCGGCG	MGGCWW1GG1	CUNTACTION	CACCATTTCA
	AGTTGATGAC	TCCGTCGGCG	TCCGTTACCA	CIMITONACI	GAGGATTICA
				********	እ ር መር እ መል ጥጥጥ
20251	GGTATTGTAC	AGTGAAGATG	TAGATATAGA	AACCCCAGAC	ACTCATATIT
	CCATAACATG	TCACTTCTAC	ATCTATATCT	TTGGGGTCTG	TGAGTATAAA
20301	CTTACATGCC	CACTATTAAG	GAAGGTAACT	CACGAGAACT	AATGGGCCAA
	GAATGTACGG	GTGATAATTC	CTTCCATTGA	GTGCTCTTGA	TTACCCGGTT
20351	CAATCTATGC	CCAACAGGCC	TAATTACATT	GCTTTTAGGG	ACAATTTTAT
	GTTAGATACG	GGTTGTCCGG	ATTAATGTAA	CGAAAATCCC	TGTTAAAATA
	0117.0	•••••			
20401	שרכיים איים	TATTACAACA	GCACGGGTAA	TATGGGTGTT	CTGGCGGGCC
20401	1GG1C1AA1G	ATAATGTTGT	CCTCCCCATT	ATACCCACAA	GACCGCCCGG
	ACCAGATTAC	MIMAIGIIGI	COIGCCCAII	AIACCCC.I.	
		GTTGAATGCT		TOCANCACAC	AAACACAGAG
20451	AAGCATCGCA	GTTGAATGCT	GIIGIAGAII	* COMMONOMO	TTTCTCTCTCTC
	TTCGTAGCGT	CAACTTACGA	CAACATCTAA	ACGLICIGIC	1110101010
					COLOCELCET
20501	CTTTCATACC	AGCTTTTGCT	TGATTCCATT	GGTGATAGAA	CCAGGIACII
	GAAAGTATGG	TCGAAAACGA	ACTAAGGTAA	CCACTATCTT	GGTCCATGAA
20551	TTCTATGTGG	AATCAGGCTG	TTGACAGCTA	TGATCCAGAT	GTTAGAATTA
	AAGATACACC	TTAGTCCGAC	AACTGTCGAT	ACTAGGTCTA	CAATCTTAAT
20601	TTGAAAATCA	TGGAACTGAA	GATGAACTTC	CAAATTACTG	CTTTCCACTG
		ACCTTGACTT			
			•		
20651	CCACCTCTCA	TTAATACAGA	GACTCTTACC	AAGGTAAAAC	CTAAAACAGG
20031	CCTCCACACT	AATTATCTCT	CTGAGAATGG	TTCCATTTTG	GATTTTGTCC
	CCICCACAC.	,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,			
20701	MC2CC2222M	CCATCCCAAA	ልልርልጥርርጥልር	AGAATTTTCA	GATAAAAATG
20/01	LAGGAAAAT	COMP COCUMA	TATOM TO THE	ጥጥተልልልልርጥ	CTATTTTTAC
	AGTCCTTTTA	CCTACCCTTT	TICINCONIG	101111111111111111111111111111111111111	~
				*****	*******
20751	AAATAAGAGT	TGGAAATAAT	TITIGCCATGG	MAAICAAICI	AAATGCCAAC
	TTTATTCTCA	ACCTTTATTA	AAACGGTACC	TTTAGTTAGA	TTTACGGTTG
					maaaaaa aa a
20801	CTGTGGAGAA	ATTTCCTGTA	CTCCAACATA	GCGCTGTATT	TGCCCGACAA
	GACACCTCTT	TAAAGGACAT	GAGGTTGTAT	CGCGACATAA	ACGGGCTGTT

Tigure 26 V

20901		CCGGGCTAGT GGCCCGATCA	
20951		TATATGGACA ATATACCTGT	
21001		CTACCGCTCA GATGGCGAGT	
21051		AGGTGCCTCA TCCACGGAGT	
21101		TCATACACCT AGTATGTGGA	
21151		GAGCTCCCTA	
21201		ATAGCATTTG TATCGTAAAC	
21251		TCCACGCTTG AGGTGCGAAC	
21301		CGACTATCTC GCTGATAGAG	
21351		CCAACGTGCC GGTTGCACGG	
21401		TGGGCCTTCA ACCCGGAAGT	
21451		CTACGACCCT GATGCTGGGA	
21501		CCTTTTACCT GGAAAATGGA	
21551		TCTGTCAGCT AGACAGTCGA	
21601		AATTAAGCGC TTAATTCGCG	
21651		TGACCAAAGA ACTGGTTTCT	
21701		TACCAGGGCT ATGGTCCCGA	
21751		 CTTTAGAAAC GAAATCTTTG	

Figure 26 W

21851	GGCATCCTAC	ACCAACACAA	CAACTCTGGA	TTTGTTGGCT	ACCTTGCCCC
	CCGTAGGATG	TGGTTGTGTT	GTTGAGACCT	AAACAACCGA	TGGAACGGGG
21901				TAACTTCCCC ATTGAAGGGG	
21951	TAGGCAAGAC	CGCAGTTGAC	AGCATTACCC	AGAAAAAGTT	TCTTTGCGAT
	ATCCGTTCTG	GCGTCAACTG	TCGTAATGGG	TCTTTTTCAA	AGAAACGCTA
22001	CGCACCCTTT	GGCGCATCCC	ATTCTCCAGT	AACTTTATGT	CCATGGGCGC
	GCGTGGGAAA	CCGCGTAGGG	TAAGAGGTCA	TTGAAATACA	GGTACCCGCG
22051	ACTCACAGAC	CTGGGCCAAA	ACCTTCTCTA	CGCCAACTCC	GCCCACGCGC
	TGAGTGTCTG	GACCCGGTTT	TGGAAGAGAT	GCGGTTGAGG	CGGGTGCGCG
22101	TAGACATGAC	TTTTGAGGTG	GATCCCATGG	ACGAGCCCAC	CCTTCTTTAT
	ATCTGTACTG	AAAACTCCAC	CTAGGGTACC	TGCTCGGGTG	GGAAGAAATA
22151	GTTTTGTTTG	AAGTCTTTGA	CGTGGTCCGT	GTGCACCAGC	CGCACCGCGG
	CAAAACAAAC	TTCAGAAACT	GCACCAGGCA	CACGTGGTCG	GCGTGGCGCC
22201	CGTCATCGAA	ACCGTGTACC	TGCGCACGCC	CTTCTCGGCC	GGCAACGCCA
	GCAGTAGCTT	TGGCACATGG	ACGCGTGCGG	GAAGAGCCGG	CCGTTGCGGT
22251	CAACATAAAG	AAGCAAGCAA	CATCAACAAC	AGCTGCCGCC	ATGGGCTCCA
	GTTGTATTTC	TTCGTTCGTT	GTAGTTGTTG	TCGACGGCGG	TACCCGAGGT
22301	GTGAGCAGGA	ACTGAAAGCC	ATTGTCAAAG	ATCTTGGTTG	TGGGCCATAT
	CACTCGTCCT	TGACTTTCGG	TAACAGTTTC	TAGAACCAAC	ACCCGGTATA
22351	TTTTTGGGCA	CCTATGACAA	GCGCTTTCCA	GGCTTTGTTT	CTCCACACAA
	AAAAACCCGT	GGATACTGTT	CGCGAAAGGT	CCGAAACAAA	GAGGTGTGTT
22401	GCTCGCCTGC	GCCATAGTCA	ATACGGCCGG	TCGCGAGACT	GGGGGCGTAC
	CGAGCGGACG	CGGTATCAGT	TATGCCGGCC	AGCGCTCTGA	CCCCCGCATG
22451	ACTGGATGGC	CTTTGCCTGG	AACCCGCACT	CAAAAACATG	CTACCTCTTT
	TGACCTACCG	GAAACGGACC	TTGGGCGTGA	GTTTTTGTAC	GATGGAGAAA
22501	GAGCCCTTTG	GCTTTTCTGA	CCAGCGACTC	AAGCAGGTTT	ACCAGTTTGA
	CTCGGGAAAC	CGAAAAGACT	GGTCGCTGAG	TTCGTCCAAA	TGGTCAAACT
22551	GTACGAGTCA	CTCCTGCGCC	GTAGCGCCAT	TGCTTCTTCC	CCCGACCGCT
	CATGCTCAGT	GAGGACGCGG	CATCGCGGTA	ACGAAGAAGG	GGGCTGGCGA
22601	GTATAACGCT	GGAAAAGTCC	ACCCAAAGCG	TACAGGGGCC	CAACTCGGCC
	CATATTGCGA	CCTTTTCAGG	TGGGTTTCGC	ATGTCCCCGG	GTTGAGCCGG
22651	GCCTGTGGAC	TATTCTGCTG	CATGTTTCTC	CACGCCTTTG	CCAACTGGCC
	CGGACACCTG	ATAAGACGAC	GTACAAAGAG	GTGCGGAAAC	GGTTGACCGG
22701	CCAAACTCCC	ATGGATCACA	ACCCCACCAT	GAACCTTATT	ACCGGGGTAC
	GGTTTGAGGG	TACCTAGTGT	TGGGGTGGTA	CTTGGAATAA	TGGCCCCATG

Figure 26 X

22801			CCTGGAGUGU GGACCTCGCG		
22851			GCGCCACTTC CGCGGTGAAG		
22901			GACACTTTCA CTGTGAAAGT		
22951			ATTTACCCC TAAATGGGGG		
23001			GCCGCGCATC CGGCGCGTAG		
23051			TTAGTGCTCC AATCACGAGG		
23101			GTTTTCACTC CAAAAGTGAG		
23151	GTTGCGCAAA	TCGTCCAGCC	GCGCCGATAT CGCGGCTATA	GAACTTCAGC	GTCAACCCCG
23201	GAGGCGGGAC	GCGCGCGCTC	TTGCGATACA AACGCTATGT	GTCCCAACGT	CGTGACCTTG
23251	TGATAGTCGC	GGCCCACCAC	CACGCTGGCC GTGCGACCGG	TCGTGCGAGA	ACAGCCTCTA
23301	GTCTAGGCGC	AGGTCCAGGA	CCGCGTTGCT GGCGCAACGA	GTCCCGCTTG	CCTCAGTTGA
23351	AACCATCGAC	GGAAGGGTTT	AAGGGCGCGT TTCCCGCGCA	CGGGTCCGAA	ACTCAACGTG
23401	AGCGTGGCAT	CACCGTAGTT	AAGGTGACCG TTCCACTGGC	ACGGGCCAGA	CCCGCAATCC
23451	TATGTCGCGG	ACGTATTTTC	• ,	GAATTTTCGG	.TGGACTCGGA
•	TTGCGCCTTC AACGCGGAAG	TCTCTTCTTG	TACGGCGTTC	TGAACGGCCT	TTTGACTAAC
	-	GGCGCAGCAC	GTGCGTCGTG	GAACGCAGCC	ACAACCTCTA
		AAAGCCGGGG	TGGCCAAGAA	GTGCTAGAAC	CGGAACGATC
23651	ACTGCTCCTT TGACGAGGAA				ATCCATTTCA TAGGTAAAGT

France 26 Y

WO 02/022080					PCT/US01/28861
23701				CCGTGTAGAC	
	TAGTGCACGA	GGAATAAATA	GTATTACGAA	GGCACATCTG	TGAATTCGAG
23751				CAACGCGCAG	
	CGGAAGCTAG	AGTUGUGTUG	CCACGTCGGT	GTTGCGCGTC	GGGCACCCGA
23801	· - · ·			ACTGCAGGTA	
	GCACTACGAA	CATCCAGTGG	AGACGTTTGC	TGACGTCCAT	GCGGACGTCC
- 23851				TTGCTGGTGA	
	TTAGCGGGGT	AGTAGCAGTG	TTTCCAGAAC	AACGACCACT	TCCAGTCGAC
23901	CAACCCGCGG	TGCTCCTCGT	TCAGCCAGGT	CTTGCATACG	GCCGCCAGAG
33701				GAACGTATGC	
23951				TCGCCTTTAG	
	GAAGGTGAAC	CAGTCCGTCA	TCAAACTTCA	AGCGGAAATC	TAGCAATAGG
24001				GCCTCCATGC	
••	TGCACCATGA	ACAGGTAGTC	GCGCGCGCGT	CGGAGGTACG	GGAAGAGGGT
24051				CATCACCGTA	
	GCGTCTGTGC	TAGCCGTGTG	AGTCGCCCAA	GTAGTGGCAT	TAAAGTGAAA
24101				GCGTCCGCAT	
	GGCGAAGCGA	CCCGAGAAGG	AGAAGGAGAA	CGCAGGCGTA	TGGTGCGCGG
24151				GTGCGCTTAC	
	TGACCCAGCA	GAAGTAAGTC	GGCGGCGTGA	CACGCGAATG	GAGGAAACGG
24201				ACCCACCATT	
	TACGAACTAA	TCGTGGCCAC	CCAACGACTT	TGGGTGGTAA	ACATCGCGGT
24251				TTACCTCTGG	
	GTAGAAGAGA	AAGAAGGAGC	GACAGGTGCT	AATGGAGACC	ACTACCGCCC
24301				TTCTTCTTGG	
	GCGAGCCCGA	ACCCTCTTCC	CGCGAAGAAA	AAGAAGAACC	CGCGTTACCG
24351				GCTGGGTGTG	
	GTTTAGGCGG	CGGCTCCAGC	TACCGGCGCC	CGACCCACAC	GCGCCGTGGT
24401				CGGACTCGAT	
	CGCGCAGAAC	ACTACTCAGA	AGGAGCAGGA	GCCTGAGCTA	TGCGGCGGAG
24451				GGCGGCGACG	
	TAGGCGAAAA	AACCCCCGCG	GCCCCCTCCG	CCGCCGCTGC	CCCTGCCCCT
24501				CGCCGCACCG	
	GCTGTGCAGG	AGGTACCAAC	CCCCTGCAGC	GCGGCGTGGC	GCAGGCGCGA
24551				GACTGGCCAT	
	GCCCCACCA	AAGCGCGACG	AGGAGAAGGG	CTGACCGGTA	AAGGAAGAGG
24601				GAGAAGAAGG	
	ATATCCGTCT	TTTTCTAGTA	CCTCAGTCAG	CTCTTCTTCC	TGTCGGATTG

Figure 262

24701	CTACCACCTT GATGGTGGAA	CCCCGTCGAG GGGGCAGCTC		
24751		ACCCAGGTTT TGGGTCCAAA	 -	
24801		GATAAAAAGC CTATTTTTCG		
24851		GCGGGGGAC CGCCCCCTG		
24901		TGTTGAAGCA ACAACTTCGT		
24951		GAGCGCAGCG CTCGCGTCGC		
25001		ACGCCACCTA TGCGGTGGAT		
25051		CATGCGAGCC GTACGCTCGG		
25101		GAGGTGCTTG CTCCACGAAC		
25151		ATCCTGCCGT TAGGACGGCA		
25201		AGGGCGCTGT TCCCGCGACA		
25251		TTTGAGGGTC AAACTCCCAG		
25301		GGAAAACAGC CCTTTTGTCG		
25351		GTGACAACGC CACTGTTGCG		
25401	GGTCACCCAC CCAGTGGGTG	TTTGCCTACC AAACGGATGG		
25,451	GCACAGTCAT CGTGTCAGTA	GAGTGAGCTG CTCACTCGAC		
25501	GATGCAAATT CTACGTTTAA	TGCAAGAACA ACGTTCTTGT		
25551	CGAGCAGCTA GCTCGTCGAT	GCGCGCTGGC CGCGCGACCG	•	

7 igure 26 AA

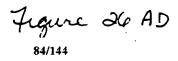
25651	TGCATGCAGC	GGTTCTTTGC	TGACCCGGAG	ATGCAGCGCA	AGCTAGAGGA
	ACGTACGTCG	CCAAGAAACG	ACTGGGCCTC	TACGTCGCGT	TCGATCTCCT
25701				CGTACGCCAG GCATGCGGTC	
25751	TCTCCAACGT	GGAGCTCTGC	AACCTGGTCT	CCTACCTTGG	AATTTTGCAC
	AGAGGTTGCA	CCTCGAGACG	TTGGACCAGA	GGATGGAACC	TTAAAACGTG
25801				TCCACGCTCA AGGTGCGAGT	
25851	GCGCCGCGAC	TACGTCCGCG	ACTGCGTTTA	CTTATTTCTA	TGCTACACCT
	CGCGGCGCTG	ATGCAGGCGC	TGACGCAAAT	GAATAAAGAT	ACGATGTGGA
25901	GGCAGACGGC	CATGGGCGTT	TGGCAGCAGT	GCTTGGAGGA	GTGCAACCTC
	CCGTCTGCCG	GTACCCGCAA	ACCGTCGTCA	CGAACCTCCT	CACGTTGGAG
25951				TTGAAGGACC AACTTCCTGG	
26001	CTTCAACGAG	CGCTCCGTGG	CCGCGCACCT	GGCGGACATC	ATTTTCCCCG
	GAAGTTGCTC	GCGAGGCACC	GGCGCGTGGA	CCGCCTGTAG	TAAAAGGGGC
26051				TGCCAGACTT ACGGTCTGAA	
26101				CTAGAGCGCT GATCTCGCGA	
26151				CTTTGTGCCC GAAACACGGG	
26201	GCGAATGCCC	TCCGCCGCTT	TGGGGCCACT	GCTACCTTCT	GCAGCTAGCC
	CGCTTACGGG	AGGCGGCGAA	ACCCCGGTGA	CGATGGAAGA	CGTCGATCGG
26251				GAAGACGTGA CTTCTGCACT	
26301	TCTACTGGAG	TGTCACTGTC	GCTGCAACCT	ATGCACCCCG	CACCGCTCCC
	AGATGACCTC	ACAGTGACAG	CGACGTTGGA	TACGTGGGGC	GTGGCGAGGG
		AAGCGTCGAC	GAATTGCTTT	CAGTTTAATA	GCCATGGAAA
26401	GAGCTGCAGG	GTCCCTCGCC	TGACGAAAAG	TCCGCGGCTC	CGGGGTTGAA
	CTCGACGTCC	CAGGGAGCGG	ACTGCTTTTC	AGGCGCCGAG	GCCCCAACTT
26451	ACTCACTCCG	GGGCTGTGGA	CGTCGGCTTA	CCTTCGCAAA	TTTGTACCTG
	TGAGTGAGGC	CCCGACACCT	GCAGCCGAAT	GGAAGCGTTT	AAACATGGAC
26501	AGGACTACCA	CGCCCACGAG	ATTAGGTTCT	ACGAAGACCA	ATCCCGCCCG
	TCCTGATGGT	GCGGGTGCTC	TAATCCAAGA	TGCTTCTGGT	TAGGGCGGGC

Figure 26 AB

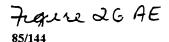
WO 02/022080					PCT/US01/28861
26551	CCTAATGCGG	AGCTTACCGC	CTGCGTCATT	ACCCAGGGCC	ACATTCTTGG
20332	• • • • • • • • • •			TGGGTCCCGG	
26601	CCAATTGCAA	GCCATCAACA	AAGCCCGCCA	AGAGTTTCTG	CTACGAAAGG
	GGTTAACGTT	CGGTAGTTGT	TTCGGGCGGT	TCTCAAAGAC	GATGCTTTCC
26651	GACGGGGGGT	TTACTTGGAC	CCCCAGTCCG	GCGAGGAGCT	CAACCCAATC
	010000000			CGCTCCTCGA	
26701	CCCCGCCGC				
	GGGGGCGCG	GCGTCGGGAT	AGTCGTCGTC	GCGCCCGGG	AACGAAGGT
26751	GGATGGCACC	CAAAAAGAAG	CTGCAGCTGC	CGCCGCCACC	CACGGACGAG
				GCGGCGTGG	
26801	GAGGAATACT				
				CAAAACCTGC	
26851	GGACATGATG			CGAGGAAGCT	
26001	AAGAGGTGTC		•		
26901				GCCAGCGTAA	
	•		•		
26951	GCGCCCCAGA				
				TACCGATGTT	
27001	TCAGGCGCCG				
				TGGGTTGGCA	
27051	CCACTGGAAC				
				TCGGCGGCGG	
27101	GAGCAACAAC				
				ACCGCGCCCG	
27151	CATAGTTGCT	-			
				GTTGTAGAGG	
27201	GCTTTCTTCT				
	CGAAAGAAGA		-		
· 27251	TACTACCGTC				
	ATGATGGCAG			•	
27301	CAGCAGCGGC				
	GTCGTCGCCG	GTGTGTCTTC	GTTTCCGCTG	GCCTATCGTT	CTGAGACTGT
27351	AAGCCCAAGA				
	TTCGGGTTCT	TTAGGTGTCG	CCGCCGTCGT	CGTCCTCCTC	CTCGCGACGC
27401	TCTGGCGCCC	AACGAACCCG	TATCGACCCG	CGAGCTTAGA	AACAGGATTT
	AGACCGCGGG	TTGCTTGGGC	ATAGCTGGGC	GCTCGAATCT	TTGTCCTAAA
27451	TTCCCACTCT	GTATGCTATA	TTTCAACAGA	GCAGGGGCCA	AGAACAAGAG
	AAGGGTGAGA	CATACGATAT	AAAGTTGTCT	CGTCCCCGGT	TCTTGTTCTC

7,gure 26 AC 83/144

27551	TCACAAAAGC	GAAGATCAGC CTTCTAGTCG	TTCGGCGCAC AAGCCGCGTG	GCTGGAAGAC CGACCTTCTG	GCGGAGGCTC CGCCTCCGAG
27601			CTGACTCTTA		
27601	AGAAGTCATT	TATGACGCGC	GACTGAGAAT	TCCTGATCAA	AGCGCGGGAA
27651	TCTCAAATTT	AAGCGCGAAA	ACTACGTCAT TGATGCAGTA	CTCCAGCGGC	CACACCCGGC GTGTGGGCCG
	AGAGIIIAAA	1100000111	101100110111		
27701	GCCAGCACCT	GTTGTCAGCG	CCATTATGAG GGTAATACTC	CAAGGAAATT	CCCACGCCCT GGGTGCGGGA
	ACATGTGGAG				
27751	TGTACACCTC	AATGGTCGGT	GTTTACCCTG	AACGCCGACC	TCGACGGGTT
27801	GACTACTCAA	CCCGAATAAA	CTACATGAGC GATGTACTCG	GCGGGACCCC	ACATGATATC TGTACTATAG
			CCCACCGAAA		
27851	GGCCCAGTTG	CCTTATGCGC	GGGTGGCTTT	GGCTTAAGAG	GACCTTGTCC
27901	CGGCTATTAC	CACCACACCT	CGTAATAACC GCATTATTGG	TTAATCCCCG	TAGTTGGCCC ATCAACCGGG
			AAGTCCCGCT		
27951	CGACGGGACC	ACATGGTCCT	TTCAGGGCGA	GGGTGGTGAC	ACCATGAAGG
28001-	CAGAGACGCC	CAGGCCGAAG	TTCAGATGAC AAGTCTACTG	TAACTCAGGG	GCGCAGCTTG CGCGTCGAAC
00053			GTGCGGTCGC		
28051	GCCCGCCGAA	AGCAGTGTCC	CACGCCAGCG	GGCCCGTCCC	ATATTGAGTG
28101	CTGACAATCA	GAGGGCGAGG	TATTCAGCTC ATAAGTCGAG	AACGACGAGT TTGCTGCTCA	CGGTGAGCTC GCCACTCGAG
			ACGGGACATT		
28151	GAGCGAACCA	GAGGCAGGCC	TGCCCTGTAA	AGTCTAGCCG	CCGCGGCCGG
28201	GCTCTTCATT	CACGCCTCGT	CAGGCAATCC	TAACTCTGCA ATTGAGACGT	GACCTCGTCC CTGGAGCAGG
20251					TTGAGGAGTT
20231	AGACTCGGCG	CGAGACCTCC	GTAACCTTGA	GACGTTAAAT	AACTCCTCAA
28301	TGTGCCATCG	GTCTACTTTA	ACCCCTTCTC	GGGACCTCCC	GGCCACTATC CCGGTGATAG
20251					GGCGGACGGC
4 0331	GCCTAGTTAA	ATAAGGATTG	AAACTGCGCC	ATTTCCTGAG	CCGCCTGCCG
28401	TACGACTGAA	TGTTAAGTGG	AGAGGCAGAG	CAACTGCGCC	TGAAACACCT ACTTTGTGGA
	MIGCIGNCII	MANAGE A CONC.			



WO 02/022080 PCT/US01/28861 28451 GGTCCACTGT CGCCGCCACA AGTGCTTTGC CCGCGACTCC GGTGAGTTTT CCAGGTGACA GCGGCGGTGT TCACGAAACG GGCGCTGAGG CCACTCAAAA 28501 GCTACTITGA ATTGCCCGAG GATCATATCG AGGGCCCGGC GCACGGCGTC CGATGAAACT TAACGGGCTC CTAGTATAGC TCCCGGGCCG CGTGCCGCAG 28551 CGGCTTACCG CCCAGGGAGA GCTTGCCCGT AGCCTGATTC GGGAGTTTAC GCCGAATGGC GGGTCCCTCT CGAACGGGCA TCGGACTAAG CCCTCAAATG 28601 CCAGCGCCC CTGCTAGTTG AGCGGGACAG GGGACCCTGT GTTCTCACTG GGTCGCGGG GACGATCAAC TCGCCCTGTC CCCTGGGACA CAAGAGTGAC 28651 TGATTTGCAA CTGTCCTAAC CCTGGATTAC ATCAAGATCT TTGTTGCCAT ACTAAACGTT GACAGGATTG GGACCTAATG TAGTTCTAGA AACAACGGTA 28701 CTCTGTGCTG AGTATAATAA ATACAGAAAT TAAAATATAC TGGGGCTCCT GAGACACGAC TCATATTATT TATGTCTTTA ATTTTATATG ACCCCGAGGA 28751 ATCGCCATCC TGTAAACGCC ACCGTCTTCA CCCGCCCAAG CAAACCAAGG TAGCGGTAGG ACATTTGCGG TGGCAGAAGT GGGCGGGTTC GTTTGGTTCC 28801 CGAACCTTAC CTGGTACTTT TAACATCTCT CCCTCTGTGA TTTACAACAG GCTTGGAATG GACCATGAAA ATTGTAGAGA GGGAGACACT AAATGTTGTC 28851 TTTCAACCCA GACGGAGTGA GTCTACGAGA GAACCTCTCC GAGCTCAGCT AAAGTTGGGT CTGCCTCACT CAGATGCTCT CTTGGAGAGG CTCGAGTCGA 28901 ACTCCATCAG AAAAAACACC ACCCTCCTTA CCTGCCGGGA ACGTACGAGT TGAGGTAGTC TTTTTTGTGG TGGGAGGAAT GGACGGCCCT TGCATGCTCA 28951 GCGTCACCGG CCGCTGCACC ACACCTACCG CCTGACCGTA AACCAGACTT CGCAGTGGCC GGCGACGTGG TGTGGATGGC GGACTGGCAT TTGGTCTGAA 29001 TTTCCGGACA GACCTCAATA ACTCTGTTTA CCAGAACAGG AGGTGAGCTT AAAGGCCTGT CTGGAGTTAT TGAGACAAAT GGTCTTGTCC TCCACTCGAA 29051 AGAAAACCT TAGGGTATTA GGCCAAAGGC GCAGCTACTG TGGGGTTTAT TCTTTTGGGA ATCCCATAAT CCGGTTTCCG CGTCGATGAC ACCCCAAATA 29101 GAACAATTCA AGCAACTCTA CGGGCTATTC TAATTCAGGT TTCTCTAGAA CTTGTTAAGT TCGTTGAGAT GCCCGATAAG ATTAAGTCCA AAGAGATCTT 29151 TCGGGGTTGG GGTTATTCTC TGTCTTGTGA TTCTCTTTAT TCTTATACTA AGCCCCAACC CCAATAAGAG ACAGAACACT AAGAGAAATA AGAATATGAT 29201 ACGCTTCTCT GCCTAAGGCT CGCCGCCTGC TGTGTGCACA TTTGCATTTA TGCGAAGAGA CGGATTCCGA GCGGCGGACG ACACACGTGT AAACGTAAAT 29251 TTGTCAGCTT TTTAAACGCT GGGGTCGCCA CCCAAGATGA TTAGGTACAT AACAGTCGAA AAATTTGCGA CCCCAGCGGT GGGTTCTACT AATCCATGTA 29301 AATCCTAGGT TTACTCACCC TTGCGTCAGC CCACGGTACC ACCCAAAAGG TTAGGATCA AATGAGTGGG AACGCAGTCG GGTGCCATGG TGGGTTTTCC 29351 TGGATTTTAA GGAGCCAGCC TGTAATGTTA CATTCGCAGC TGAAGCTAAT ACCTAAAATT CCTCGGTCGG ACATTACAAT GTAAGCGTCG ACTTCGATTA



29451	TCGCCACAAA AGCGGTGTTT	AACAAAATTG TTGTTTTAAC	GCAAGTATGC CGTTCATACG	TGTTTATGCT ACAAATACGA	ATTTGGCAGC TAAACCGTCG
29501	CAGGTGACAC GTCCACTGTG	TACAGAGTAT ATGTCTCATA	AATGTTACAG TTACAATGTC	TTTTCCAGGG AAAAGGTCCC	TAAAAGTCAT ATTTTCAGTA
29551	AAAACTTTTA TTTTGAAAAT	TGTATACTTT ACATATGAAA	TCCATTTTAT AGGTAAAATA	GAAATGTGCG CTTTACACGC	ACATTACCAT TGTAATGGTA
29601		AAACAGTATA TTTGTCATAT			
29651	ACACTGGCAC TGTGACCGTG	TTTCTGCTGC AAAGACGACG	ACTGCTATGC TGACGATACG	TAATTACAGT ATTAATGTCA	GCTCGCTTTG CGAGCGAAAC
29701		TACTCTATAT ATGAGATATA			
29751		ATGCCTTAAT TACGGAATTA			
29801	AACTGCTTTA TTGACGAAAT	CTCGCTGCTT GAGCGACGAA	GCAAAACAAA CGTTTTGTTT	TTCAAAAAGT AAGTTTTTCA	TAGCATTATA ATCGTAATAT
29851		GATTTAAACC CTAAATTTGG			
29901	GGACTTGTTA	TGACTCTATG ACTGAGATAC	ACCCTATACG	AGGTCGCGAT	GTTGGAACTT
29951	CAGTCCGAAG	CTGGATGTCA GACCTACAGT	CGTAGACTGA	AACCGGTCGT	GGACAGGGCG
30001	CCTAAACAAG	CAGTCCAACT GTCAGGTTGA	TGTCGCTGGG	TGGGATTGTC	TCTACTGGTT
30051	GTGTTGGTTG	CGCCGCCGC	GATGGCCTGA	ATGTAGATGG	TGTTTATGTG
30101	GGGTTCAAAG	TGCCTTTGTC ACGGAAACAG	TTATTGACCC	TATTGAACCC	GTACACCACC
	AAGAGGTATC	CGCTTATGTT GCGAATACAA	ACATACGGAA	TAATAATACA	CCGAGTAGAC
	GACGGATTTC	CGCAAACGCG GCGTTTGCGC	GGGCTGGTGG	GTAGATATCA	GGGTAGTAAC
	ACGATGTGGG	AAACAATGAT TTTGTTACTA	CCTTAGGTAT	CTAACCTGCC	TGACTTTGTG
30301	ATGTTCTTTT TACAAGAAAA	CTCTTACAGT GAGAATGTCA	ATGATTAAAT TACTAATTTA	GAGACATGAT CTCTGTACTA	AGGAGCTCAA

Figure 26 AF

30401			 TCCAGCCTTC AGGTCGGAAG	
30451			 TCTGCAGCCT AGACGTCGGA	
30501			 GTCTGTGTGC CAGACACACG	
.30551			GACTATAGCT CTGATATCGA	
30601			TTTTCTGCTG AAAAGACGAC	
30651			AGCCTCAAAG TCGGAGTTTC	
30701		-	 AGTTGCTACA TCAACGATGT	
30751			 CATCTCTGTT GTAGAGACAA	
30801	•		 CCTACCTTGA GGATGGAACT	
30851			TTCCCCGCGC AAGGGGCGCG	
30901			 TGTCCCAGCC ACAGGGTCGG	
30951			GCTACTTTAA CGATGAAATT	
31001			GGACGGAATT CCTGCCTTAA	
31051			 CCGAGCAACA GGCTCGTTGT	
31101	CAAGAGCTCC GTTCTCGAGG		CAGTGCAAAA GTCACGTTTT	
31151	TTGTCTCGTA AACAGAGCAT			
31201	ACCGCCTTAG TGGCGGAATC		GTCAGAAATT CAGTCTTTAA	
31251	GTGGGAGAAA CACCCTCTTT		CACTCGGTAG GTGAGCCATC	

Figure 26 A6 87/144

31351					ATAAAAAAAA TTTTTTTTAT
31401				TAGCAAATTT ATCGTTTAAA	
31451				AGCTCTGGTA TCGAGACCAT	
31501				AATGGAATGT TTACCTTACA	
31551				CATGTTGTTG GTACAACAAC	
31601				CCGTGTATCC GGCACATAGG	
31651				ACTCCTCCCT TGAGGAGGGA	
31701				ACTCTCTTTG TGAGAGAAAC	
31751				CGCTCAAAAT GCGAGTTTTA	
31801				TCCCAAAATG AGGGTTTTAC	
31851				CATAAACCTG GTATTTGGAC	
31901				CTGTGGCTGC GACACCGACG	
31951	GATTACCAGC	GCCCGTTGTG	TGAGTGGTAC	CAATCACAGG GTTAGTGTCC	GGGGCGATTG
32001	CGTGCACGAC GCACGTGCTG	TCCAAACTTA AGGTTTGAAT	GCATTGCCAC CGTAACGGTG	CCAAGGACCC GGTTCCTGGG	CTCACAGTGT GAGTGTCACA
32051	CAGAAGGAAA GTCTTCCTTT			GCCCCCTCAC CGGGGGAGTG	
32101	AGCAGTACCC TCGTCATGGG			CCTCTAACTA GGAGATTGAT	
32151	TAGCTTGGGC ATCGAACCCG			TTATACACAA AATATGTGTT	
32201	TAGGACTAAA ATCCTGATTT			TAACAGACGA ATTGTCTGCT	

Figure 26 AH

32301	AACTAAAGTT TTGATTTCAA		 	AATATGCAAC TTATACGTTG
32351		AGGAGGACTA TCCTCCTGAT		ACGCCTTATA TGCGGAATAT
32401	•		-	ATCTAAGACT TAGATTCTGA
32451		CCTCTTTTTA GGAGAAAAAT	 	-
32501	***	CCTTTACTTG GGAAATGAAC	 	
32551	•	TAAGCACTGC ATTCGTGACG	 	
32601		GCAGGAGATG CGTCCTCTAC		
32651		CCTCAAAACA GGAGTTTTGT		
32701		TGGTTCCTAA ACCAAGGATT	 	
32751		ACAGTAGGAA TGTCATCCTT		
32801		TCCATCTCCT AGGTAGAGGA		
32851		TGGTCTTAAC ACCAGAATTG		
32901		GCTGTTAAAG CGACAATTTC		
32951		TCTTATTATA AGAATAATAT		
33001	AATTCCTTCC TTAAGGAAGG	TGGACCCAGA ACCTGGGTCT		
33051	TGAAGGCACA ACTTCCGTGT	GCCTATACAA CGGATATGTT		
33101	CTTATCCAAA GAATAGGTTT	ATCTCACGGT TAGAGTGCCA		
33151	GTTTACTTAA CAAATGAATT	ACGGAGACAA TGCCTCTGTT		

Figure 26 AI 89/144

33251	CATTTTCATG	GGACTGGTCT	GGCCACAACT	ACATTAATGA	AATATTTGCC
	GTAAAAGTAC	CCTGACCAGA	CCGGTGTTGA	TGTAATTACT	TTATAAACGG
33301	ACATCCTCTT	ACACTTTTTC	ATACATTGCC	CAAGAATAAA	GAATCGTTTG
	TGTAGGAGAA	TGTGAAAAAG	TATGTAACGG	GTTCTTATTT	CTTAGCAAAC
33351	TGTTATGTTT	CAACGTGTTT	ATTTTTCAAT	TGCAGAAAAT	TTCAAGTCAT
	ACAATACAAA	GTTGCACAAA	TAAAAAGTTA	ACGTCTTTTA	AAGTTCAGTA
33401	TTTTCATTCA	GTAGTATAGC	CCCACCACCA	CATAGCTTAT	ACAGATCACC
	AAAAGTAAGT	CATCATATCG	GGGTGGTGGT	GTATCGAATA	TGTCTAGTGG
33451	GTACCTTAAT	CAAACTCACA	GAACCCTAGT	ATTCAACCTG	CCACCTCCCT
	CATGGAATTA	GTTTGAGTGT	CTTGGGATCA	TAAGTTGGAC	GGTGGAGGGA
33501	CCCAACACAC	AGAGTACACA	GTCCTTTCTC	CCCGGCTGGC	CTTAAAAAGC
	GGGTTGTGTG	TCTCATGTGT	CAGGAAAGAG	GGGCCGACCG	GAATTTTTCG
33551	ATCATATCAT	GGGTAACAGA	CATATTCTTA	GGTGTTATAT	TCCACACGGT
	TAGTATAGTA	CCCATTGTCT	GTATAAGAAT	CCACAATATA	AGGTGTGCCA
33601	TTCCTGTCGA	GCCAAACGCT	CATCAGTGAT	ATTAATAAAC	TCCCCGGGCA
	AAGGACAGCT	CGGTTTGCGA	GTAGTCACTA	TAATTATTTG	AGGGGCCCGT
33651	GCTCACTTAA	GTTCATGTCG	CTGTCCAGCT	GCTGAGCCAC	AGGCTGCTGT
	CGAGTGAATT	CAAGTACAGC	GACAGGTCGA	CGACTCGGTG	TCCGACGACA
33701	CCAACTTGCG	GTTGCTTAAC	GGGCGGCGAA	GGAGAAGTCC	ACGCCTACAT
	GGTTGAACGC	CAACGAATTG	CCCGCCGCTT	CCTCTTCAGG	TGCGGATGTA
33751	GGGGGTAGAG	TCATAATCGT	GCATCAGGAT	AGGGCGGTGG	TGCTGCAGCA
	CCCCCATCTC	AGTATTAGCA	CGTAGTCCTA	TCCCGCCACC	ACGACGTCGT
33801	CGCGCGCTTA	TTTGACGACG	CĠCCGCCGCT GCGGCGGCGA	GGCAGGACGT	CCTTATGTTG
33851	TACCGTCACC	AGAGGAGTCG	GATGATTCGC CTACTAAGCG	TGGCGGGCGT	CGTATTCCGC
33901	GGAACAGGAG	GCCCGTGTCG		CTAGAGTGAA	TTTAGTCGTG
	TCATTGACGT	CGTGTCGTGG	TGTTATAACA	AGTTTTAGGG	ACAGTGCAAG TGTCACGTTC
	CGCGACATAG	GTTTCGAGTA	CCGCCCCTGG	TGTCTTGGGT	CGTGGCCATC
34051	ATACCACAAG TATGGTGTTC	CGCAGGTAGA GCGTCCATCT	TTAAGTGGCG AATTCACCGC	ACCCCTCATA TGGGGAGTAT	AACACGCTGG TTGTGCGACC
34101	ACATAAACAT	TACCTCTTTT	GGCATGTTGT	AATTCACCAC	CTCCCGGTAC
	TGTATTTGTA	ATGGAGAAAA	CCGTACAACA	TTAAGTGGTG	GAGGGCCATG

Figure 26 AJ

34201				CTGCAGGGAA GACGTCCCTT	
34251				AACCATGGAT TTGGTACCTA	
34301				CACACGTGCA GTGTGCACGT	
34351				CATATCCCAG GTATAGGGTC	
34401				AGGGAAGACC TCCCTTCTGG	
34451		*	· • · ·	TCGGGCAGCA AGCCCGTCGT	
34501	•••			AAAAGGAGGT TTTTCCTCCA	
34551	•			ATCGTGTTGG TAGCACAACC	
34601				TTTCCTGAAG AAAGGACTTC	
34651				GGTCTCGCCG CCAGAGCGGC	
34701			-	CTCAAAGCAT GAGTTTCGTA	
34751	•			ATGCGCCGCT TACGCGGCGA	
34801				GCCAACCTAC CGGTTGGATG	
34851				GCTGGAAGAA CGACCTTCTT	
	AAAAAATAAG	GTTTTCTAAT	AGGTTTTGGA	CAAAATGAAG GTTTTACTTC	TAGATAATTC
	ACTTGCGCGA	GGGGAGGCCA	CCGCACCAGT	AACTCTACAG TTGAGATGTC	GGTTTCTTGT
35001	CTATTACCGT	AAACATTCTA	CAACGTGTTA	GGCTTCCAAA CCGAAGGTTT	TCCGTTTGCC
35051	••••			ACCCTTCAGG TGGGAAGTCC	•

Figure 26 AK

PCT/US01/28861 WO 02/022080

35151	CCACCTTCTC	AATATATCTC	TAAGCAAATC	CCGAATATTA	AGTCCGGCCA
	GGTGGAAGAG	TTATATAGAG	ATTCGTTTAG	GGCTTATAAT	TCAGGCCGGT
35201	TTGTAAAAAT	CTGCTCCAGA	GCGCCCTCCA	CCTTCAGCCT	CAAGCAGCGA
	AACATTTTTA	GACGAGGTCT	CGCGGGAGGT	GGAAGTCGGA	GTTCGTCGCT
35251	ATCATGATTG	CAAAAATTCA	GGTTCCTCAC	AGACCTGTAT	AAGATTCAAA
	TAGTACTAAC	GTTTTTAAGT	CCAAGGAGTG	TCTGGACATA	TTCTAAGTTT
35301	AGCGGAACAT	ТААСААААТ	ACCGCGATCC	CGTAGGTCCC	TTCGCAGGGC
	TCGCCTTGTA	АТТСТТТТА	TGGCGCTAGG	GCATCCAGGG	AAGCGTCCCG
35351	CAGCTGAACA	TAATCGTGCA	GGTCTGCACG	GACCAGCGCG	GCCACTTCCC
	GTCGACTTGT	ATTAGCACGT	CCAGACGTGC	CTGGTCGCGC	CGGTGAAGGG
35401	CGCCAGGAAC	CATGACAAAA	GAACCCACAC	TGATTATGAC	ACGCATACTC
	GCGGTCCTTG	GTACTGTTTT	CTTGGGTGTG	ACTAATACTG	TGCGTATGAG
35451	GGAGCTATGC	TAACCAGCGT	AGCCCCGATG	TAAGCTTGTT	GCATGGGCGG
	CCTCGATACG	ATTGGTCGCA	TCGGGGCTAC	ATTCGAACAA	CGTACCCGCC
35501	CGATATAAAA	TGCAAGGTGC	TGCTCAAAAA	ATCAGGCAAA	GCCTCGCGCA
	GCTATATTTT	ACGTTCCACG	ACGAGTTTTT	TAGTCCGTTT	CGGAGCGCGT
35551	AAAAAGAAAG	CACATCGTAG	TCATGCTCAT	GCAGATAAAG	GCAGGTAAGC
	TTTTTCTTTC	GTGTAGCATC	AGTACGAGTA	CGTCTATTTC	CGTCCATTCG
35601	TCCGGAACCA	CCACAGAAAA	AGACACCATT	TTTCTCTCAA	ACATGTCTGC
	AGGCCTTGGT	GGTGTCTTTT	TCTGTGGTAA	AAAGAGAGTT	TGTACAGACG
35651	GGGTTTCTGC	ATAAACACAA	AATAAAATAA	CAAAAAAACA	TTTAAACATT
	CCCAAAGACG	TATTTGTGTT	TTATTTTATT	GTTTTTTTGT	AAATTTGTAA
35701	AGAAGCCTGT	CTTACAACAG	GAAAAACAAC	CCTTATAAGC	ATAAGACGGA
	TCTTCGGACA	GAATGTTGTC	CTTTTTGTTG	GGAATATTCG	TATTCTGCCT
35751	CTACGGCCAT	GCCGGCGTGA	CCGTAAAAAA	ACTGGTCACC	GTGATTAAAA
	GATGCCGGTA	CGGCCGCACT	GGCATTTTT	TGACCAGTGG	CACTAATTTT
35801	AGCACCACCG	ACAGCTCCTC	GGTCATGTCC	GGAGTCATAA	TGTAAGACTC
	TCGTGGTGGC	TGTCGAGGAG	CCAGTACAGG	CCTCAGTATT	ACATTCTGAG
35851	GGTAAACACA	TCAGGTTGAT	TCACATCGGT	CAGTGCTAAA	AAGCGACCGA
	CCATTTGTGT	AGTCCAACTA	AGTGTAGCCA	GTCACGATTT	TTCGCTGGCT
35901	AATAGCCCGG	GGGAATACAT	ACCCGCAGGC	GTAGAGACAA	CATTACAGCC
	TTATCGGGCC	CCCTTATGTA	TGGGCGTCCG	CATCTCTGTT	GTAATGTCGG
35951	CCCATAGGAG	GTATAACAAA	ATTAATAGGA	GAGAAAAACA	CATAAACACC
	GGGTATCCTC	CATATTGTTT	TAATTATCCT	CTCTTTTTGT	GTATTTGTGG
36001	TGAAAAACCC	TCCTGCCTAG	GCAAAATAGC	ACCCTCCCGC	TCCAGAACAA
	ACTTTTTGGG	AGGACGGATC	CGTTTTATCG	TGGGAGGGCG	AGGTCTTGTT

Figure 26 AL

36101	AAAGAAAACC TTTCTTTTGG		ACACGGCACC TGTGCCGTGG	
36151			GCGAGTATAT CGCTCATATA	
36201			AACACCCAGA TTGTGGGTCT	
36251			AACCCACAAC TTGGGTGTTG	
36301			TTCCCATTTT AAGGGTAAAA	
36351			CTAAAACCTA GATTTTGGAT	
36401			ACTCCACCC TGAGGTGGGG	
				PacI
34454		 		
36451		 	ATTGATGATG TAACTACTAC	
36501			CTTCCCCATT GAAGGGGTAA	
36551			TGCAGGCCAT ACGTCCGGTA	
36601			CAAGGCCAGC GTTCCGGTCG	
36651			TTTCCATAGG AAAGGTATCC	
36701			GTCAGAGGTG CAGTCTCCAC	
36751	ACAGGACTAT TGTCCTGATA		CCTGGAAGCT GGACCTTCGA	
36801	CTCTCCTGTT GAGAGGACAA		ATACCTGTCC TATGGACAGG	
36851	CTTCGGGAAG GAAGCCCTTC	_	CACGCTGTAG GTGCGACATC	
36901	TCGGTGTAGG AGCCACATCC	 	TGTGTGCACG ACACACGTGC	

Figure 26 AM

37001	CGGTAAGACA	CGACTTATCG	CCACTGGCAG	CAGCCACTGG	TAACAGGATT
	GCCATTCTGT	GCTGAATAGC	GGTGACCGTC	GTCGGTGACC	ATTGTCCTAA
37051	AGCAGAGCGA	GGTATGTAGG	CGGTGCTACA	GAGTTCTTGA	AGTGGTGGCC
	TCGTCTCGCT	CCATACATCC	GCCACGATGT	CTCAAGAACT	TCACCACCGG
37101	TAACTACGGC	TACACTAGAA	GGACAGTATT	TGGTATCTGC	GCTCTGCTGA
	ATTGATGCCG	ATGTGATCTT	CCTGTCATAA	ACCATAGACG	CGAGACGACT
37151	AGCCAGTTAC	CTTCGGAAAA	AGAGTTGGTA	GCTCTTGATC	CGGCAAACAA
	TCGGTCAATG	GAAGCCTTTT	TCTCAACCAT	CGAGAACTAG	GCCGTTTGTT
37201		GTAGCGGTGG CATCGCCACC			
37251	CAGAAAAAA	GGATCTCAAG	AAGATCCTTT	GATCTTTTCT	ACGGGGTCTG
	GTCTTTTTT	CCTAGAGTTC	TTCTAGGAAA	CTAGAAAAGA	TGCCCCAGAC
37301	ACGCTCAGTG	GAACGAAAAC	TCACGTTAAG	GGATTTTGGT	CATGAGATTA
	TGCGAGTCAC	CTTGCTTTTG	AGTGCAATTC	CCTAAAACCA	GTACTCTAAT
37351	TCAAAAAGGA	TCTTCACCTA	GATCCTTTTA	AATCAATCTA	AAGTATATAT
	AGTTTTTCCT	AGAAGTGGAT	CTAGGAAAAT	TTAGTTAGAT	TTCATATATA
37401		GGTCTGACAG CCAGACTGTC			
37451	CTCAGCGATC	TGTCTATTTC	GTTCATCCAT	AGTTGCCTGA	CTCCCCGTCG
	GAGTCGCTAG	ACAGATAAAG	CAAGTAGGTA	TCAACGGACT	GAGGGGCAGC
37501	TGTAGATAAC	TACGATACGG	GAGGGCTTAC	CATCTGGCCC	CAGTGCTGCA
	ACATCTATTG	ATGCTATGCC	CTCCCGAATG	GTAGACCGGG	GTCACGACGT
37551	ATGATACCGC	GAGACCCACG	CTCACCGGCT	CCAGATTTAT	CAGCAATAAA
	TACTATGGCG	CTCTGGGTGC	GAGTGGCCGA	GGTCTAAATA	GTCGTTATTT
37601	CCAGCCAGCC	GGAAGGGCCG	AGCGCAGAAG	TGGTCCTGCA	ACTTTATCCG
	GGTCGGTCGG	CCTTCCCGGC	TCGCGTCTTC	ACCAGGACGT	TGAAATAGGC
37651	CCTCCATCCA	GTCTATTAAT	TGTTGCCGGG	AAGCTAGAGT	AAGTAGTTCG
	GGAGGTAGGT	CAGATAATTA	ACAACGGCCC	TTCGATCTCA	TTCATCAAGC
37701	CCAGTTAATA	GTTTGCGCAA	CGTTGTTGCC	ATTGCTACAG	GCATCGTGGT
	GGTCAATTAT	CAAACGCGTT	GCAACAACGG	TAACGATGTC	CGTAGCACCA
37751	GTCACGCTCG	TCGTTTGGTA	TGGCTTCATT	CAGCTCCGGT	TCCCAACGAT
	CAGTGCGAGC	AGCAAACCAT	ACCGAAGTAA	GTCGAGGCCA	AGGGTTGCTA
37801	CAAGGCGAGT	TACATGATCC	CCCATGTTGT	GCAAAAAAGC	GGTTAGCTCC
	GTTCCGCTCA	ATGTACTAGG	GGGTACAACA	CGTTTTTTCG	CCAATCGAGG
37851	TTCGGTCCTC	CGATCGTTGT	CAGAAGTAAG	TTGGCCGCAG	TGTTATCACT
	AAGCCAGGAG	GCTAGCAACA	GTCTTCATTC	AACCGGCGTC	ACAATAGTGA

Figure 26 AN

37951	GATGCTTTTC CTACGAAAAG		GAGTACTCAA CTCATGAGTT		
38001	TGTATGCGGC ACATACGCCG		CTCTTGCCCG GAGAACGGGC		
38051			TAAAAGTGCT ATTTTCACGA		
38101			ATCTTACCGC TAGAATGGCG		
38151	TAACCCACTC ATTGGGTGAG	GTGCACCCAA CACGTGGGTT	CTGATCTTCA GACTAGAAGT	GCATCTTTTA CGTAGAAAAT	CTTTCACCAG GAAAGTGGTC
38201			CAGGAAGGCA GTCCTTCCGT		
38251			TGAATACTCA ACTTATGAGT		
38301			TTATTGTCTC AATAACAGAG		
38351			AAATAGGGGT TTTATCCCCA		
38401	AAGTGCCACC TTCACGGTGG		GAAACCATTA CTTTGGTAAT		
38451	AAAAATAGGC TTTTTTATCCG		GCCCTTTCGT CGGGAAAGCA		
		PacI			
	TTCTTAATTT AAGAATTAAA				

Figure 26 AD

1	CATCATCAAT	AATATACCTT	ATTTTGGATT	GAAGCCAATA	TGATAATGAG
	GTAGTAGTTA	TTATATGGAA	TAAAACCTAA	CTTCGGTTAT	ACTATTACTC
	•				
51	GGGGTGGAGT	TTGTGACGTG	GCGCGGGGCG	TGGGAACGGG	GCGGGTGACG
	CCCCACCTCA	AACACTGCAC	CGCGCCCCGC	ACCCTTGCCC	CGCCCACTGC
101	TAGTAGTGTG	GCGGAAGTGT	GATGTTGCAA	GTGTGGCGGA	ACACATGTAA
	ATCATCACAC	CGCCTTCACA	CTACAACGTT	CACACCGCCT	TGTGTACATT
				•	
151	GCGACGGATG	TGGCAAAAGT	GACGTTTTTG	GTGTGCGCCG	GTGTACACAG
	CGCTGCCTAC	ACCGTTTTCA	CTGCAAAAAC	CACACGCGGC	CACATGTGTC
201	GAAGTGACAA	TTTTCGCGCG	GTTTTAGGCG	GATGTTGTAG	TAAATTTGGG
	CTTCACTGTT	AAAAGCGCGC	CAAAATCCGC	CTACAACATC	ATTTAAACCC
•					
251	CGTAACCGAG	TAAGATTTGG	CCATTTTCGC	GGGAAAACTG	AATAAGAGGA
	GCATTGGCTC	ATTCTAAACC	GGTAAAAGCG	CCCTTTTGAC	TTATTCTCCT
301	AGTGAAATCT	GAATAATTTT	GTGTTACTCA	TAGCGCGTAA	TATTTGTCTA
	TCACTTTAGA	CTTATTAAAA	CACAATGAGT	ATCGCGCATT	ATAAACAGAT
				•	
351	GGGCCGCGG	GACTTTGACC	GTTTACGTGG	AGACTCGCCC	AGGTGTTTTT
	CCCGGCGCCC	CTGAAACTGG	CAAATGCACC	TCTGAGCGGG	TCCACAAAAA
401	CTCAGGTGTT	TTCCGCGTTC	CGGGTCAAAG	TTGGCGTTTT	ATTATTATAG
	GAGTCCACAA	AAGGCGCAAG	GCCCAGTTTC	AACCGCAAAA	TAATAATATC
451	GCGGCCGCGA	TCCATTGCAT	ACGTTGTATC	CATATCATAA	TATGTACATT
	CGCCGGCGCT	AGGTAACGTA	TGCAACATAG	GTATAGTATT	ATACATGTAA
501	TATATTGGCT	CATGTCCAAC	ATTACCGCCA	TGTTGACATT	GATTATTGAC
	ATATAACCGA	GTACAGGTTG	TAATGGCGGT	ACAACTGTAA	CTAATAACTG
	•				
551	TAGTTATTAA	TAGTAATCAA	TTACGGGGTC	ATTAGTTCAT	AGCCCATATA
	ATCAATAATT	ATCATTAGTT	AATGCCCCAG	TAATCAAGTA	TCGGGTATAT
601	TGGAGTTCCG	CGTTACATAA	CTTACGGTAA	ATGGCCCGCC	TGGCTGACCG
	ACCTCAAGGC	GCAATGTATT	GAATGCCATT	TACCGGGCGG	ACCGACTGGC
651	CCCAACGACC	CCCGCCCATT	GACGTCAATA	ATGACGTATG	TTCCCATAGT
	GGGTTGCTGG	GGGCGGGTAA	CTGCAGTTAT	TACTGCATAC	AAGGGTATCA
701	AACGCCAATA	GGGACTTTCC	ATTGACGTCA	ATGGGTGGAG	TATTTACGGT
	TTGCGGTTAT	CCCTGAAAGG	TAACTGCAGT	TACCCACCTC	ATAAATGCCA
751	AAACTGCCCA	CTTGGCAGTA	CATCAAGTGT	ATCATATGCC	AAGTACGCCC
	TTTGACGGGT	GAACCGTCAT	GTAGTTCACA	TAGTATACGG	TTCATGCGGG
801	CCTATTGACG	TCAATGACGG	TAAATGGCCC	GCCTGGCATT	ATGCCCAGTA
	GGATAACTGC	AGTTACTGCC	ATTTACCGGG	CGGACCGTAA	TACGGGTCAT

Figure 27A

901	TCGCTATTAC	CATGGTGATG	CGGTTTTGGC	AGTACATCAA	TGGGCGTGGA
	AGCGATAATG	GTACCACTAC	GCCAAAACCG	TCATGTAGTT	ACCCGCACCT
951	TAGCGGTTTG	ACTCACGGGG	ATTTCCAAGT	CTCCACCCCA	TTGACGTCAA
	ATCGCCAAAC	TGAGTGCCCC	TAAAGGTTCA	GAGGTGGGGT	AACTGCAGTT
1001	TGGGAGTTTG	TTTTGGCACC	AAAATCAACG	GGACTTTCCA	AAATGTCGTA
				CCTGAAAGGT	
1051				GTAGGCGTGT	
		•		CATCCGCACA	
1101				CGTCAGATCG	
				GCAGTCTAGC	
1151				ACACCGGGAC	
				TGTGGCCCTG	
1201					TGCCAAGAGT .
				CCTAAGGGGC	
1251				CAAGAGGTCC	
				GTTCTCCAGG	
1301				CCGAGCCCGC	
				GGCTCGGGCG	
1351				GTGGGCGCCG	
1401				CACCCGCGGC	
1401				CAACACCGCC	
1451				GTTGTGGCGG	
1451				ACGAGGAGGT	
1501				TGCTCCTCCA ACCTACAAGG	
1001				TGGATGTTCC	
1551				CCTGGAGGGC	
				GGACCTCCCG	
1601				GGGTGTACCA	
				CCCACATGGT	
1651				GGCCCCGGCA	
1031					
1701				CCGGGGCCGT	
1701				GCCCGTGGAG	
				CGGGCACCTC	
				GCGCCGCCCA	
	ACCTCCTCCG	GTTGCTCCCG	CICTIGTTGA	LGCGGCGGGT	GGGGTACAGG

Figure 27B

PCT/US01/28861 WO 02/022080

1851	CTCCAAGCTG	GCCTTCCACC	ACGTGGCCAG	GGAGCTGCAC	CCCGAGTACT
	GAGGTTCGAC	CGGAAGGTGG	TGCACCGGTC	CCTCGACGTG	GGGCTCATGA
1901		CTAAAGCCCG GATTTCGGGC			
1951	CCATCTGTTG GGTAGACAAC	TTTGCCCCTC AAACGGGGAG			
2001	CACTCCCACT	GTCCTTTCCT	AATAAAATGA	GGAAATTGCA	TCGCATTGTC
	GTGAGGGTGA	CAGGAAAGGA	TTATTTTACT	CCTTTAACGT	AGCGTAACAG
2051		TCATTCTATT AGTAAGATAA			
2101		GGGAAGACAA CCCTTCTGTT			
2151		CGCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC			
2201	GGGAAAGAAT	ATATAAGGTG	GGGGTCTTAT	GTAGTTTTGT	ATCTGTTTTG
	CCCTTTCTTA	TATATTCCAC	CCCCAGAATA	CATCAAAACA	TAGACAAAAC
2251	CAGCAGCCGC	CGCCGCCATG	AGCACCAACT	CGTTTGATGG	AAGCATTGTG
	GTCGTCGGCG	GCGGCGGTAC	TCGTGGTTGA	GCAAACTACC	TTCGTAACAC
2301	AGCTCATATT	TGACAACGCG	CATGCCCCCA	TGGGCCGGGG	TGCGTCAGAA
	TCGAGTATAA	ACTGTTGCGC	GTACGGGGGT	ACCCGGCCCC	ACGCAGTCTT
2351	TGTGATGGGC	TCCAGCATTG	ATGGTCGCCC	CGTCCTGCCC	GCAAACTCTA
	ACACI ACCCG	AGGTCGTAAC	TACCAGCGGG	GCAGGACGGG	CGTTTGAGAT
2401	CTACCTTGAC	CTACGAGACC	GTGTCTGGAA	CGCCGTTGGA	GACTGCAGCC
	GATGGAACTG	GATGCTCTGG	CACAGACCTT	GCGGCAACCT	CTGACGTCGG
2451	TCCGCCGCCG	CTTCAGCCGC	TGCAGCCACC	GCCCGCGGGA	TTGTGACTGA
	AGGCGGCGGC	GAAGTCGGCG	ACGTCGGTGG	CGGGCGCCCT	AACACTGACT
2501	CTTTGCTTTC	CTGAGCCCGC	TTGCAAACAĞ	TGCAGCTTCC	CGTTCATCCG
	GAAACGAAAG	GACTCGGGCG	AACGTTTGTC	ACGTCGAAGG	GCAAGTAGGC
2551	CCCGCGATGA	CAAGTTGACG	GCTCTTTTGG	CACAATTGGA	TTCTTTGACC
	GGGCGCTACT	GTTCAACTGC	CGAGAAAACC	GTGTTAACCT	AAGAAACTGG
2601	CGGGAACTTA	ATGTCGTTTC	TCAGCAGCTG	TTGGATCTGC	GCCAGCAGGT
	GCCCTTGAAT	TACAGCAAAG	AGTCGTCGAC	AACCTAGACG	CGGTCGTCCA
2651	TTCTGCCCTG	AAGGCTTCCT	CCCCTCCCAA	TGCGGTTTAA	AACATAAATA
	AAGACGGGAC	TTCCGAAGGA	GGGGAGGGTT	ACGCCAAATT	TTGTATTTAT
2701	AAAAACCAGA	CTCTGTTTGG	ATTTGGATCA	AGCAAGTGTC	TTGCTGTCTT
	TTTTTGGTCT	GAGACAAACC	TAAACCTAGT	TCGTTCACAG	AACGACAGAA

Figure 27 C

2751				GGTCTCGGTC CCAGAGCCAG
2801		CTGTGTATTT GACACATAAA		TGACTCTGGA ACTGAGACCT
2851		CATGGGCATA GTACCCGTAT	 	
2901		CATGCTGCGG GTACGACGCC		
2951		GCGTGGTGCC CGCACCACGG	 -	· · · · · · · · · · · · · · · · · · ·
3001	• •	GCCCTTGGTG CGGGAACCAC	 	
3051		GTGGGGATAT CACCCCTATA		
3101	GGCTATGTTC CCGATACAAG	CCAGCCATAT GGTCGGTATA		
3151	CCAGCACAGT GGTCGTGTCA	GTATCCGGTG CATAGGCCAC		
3201		GGAAGAACTT CCTTCTTGAA	 	
3251		TCCATAATGA AGGTATTACT		
3301	*	TCTGGGATCA AGACCCTAGT	 	
3351		CCATTTTTAC GGTAAAAATG	 	
3401	•••	CCATCCGGCC GGTAGGCCGG	 	
	TTTCCCACGC AAAGGGTGCG		 	
3501	ATGAAGAAAA TACTTCTTTT	CGGTTTCCGG GCCAAAGGCC	 	
	GTTCCTGAGC CAAGGACTCG			
3601	CTATTACCGG GATAATGGCC	CTGCAACTGG GACGTTGACC		
3651	CTGAGCAGGG			

3701			GGCGCTCGCC CCGCGAGCGG		
3751			AACGGTTTGA TTGCCAAACT		
3801			CAGTTCCAGG GTCAAGGTCC		
3851			CCAGCATATC GGTCGTATAG		
3901			GTAGTCGGTG CATCAGCCAC		
3951			AGGGTCCTCG TCCCAGGAGC		
4001			CTGCGCGCTG GACGCGCGAC		
4051	GGTCCTGCTG CCAGGACGAC	GTGCTGAAGC CACGACTTCG	GCTGCCGGTC CGACGGCCAG	TTCGCCCTGC AAGCGGGACG	GCGTCGGCCA CGCAGCCGGT
4101			TCATAGTCCA AGTATCAGGT		
4151			GGAGGAGGCG CCTCCTCCGC		
4201			TGGGCGCGAG ACCCGCGCTC		
4251	AGGCATCCGC TCCGTAGGCG	GCCGCAGGCC CGGCGTCCGG	CCGCAGACGG GGCGTCTGCC	TCTCGCATTC AGAGCGTAAG	CACGAGCCAG GTGCTCGGTC
4301			GTCAAAAACC CAGTTTTTGG		
4351	GATGCGTTTC CTACGCAAAG	TTACCTCTGG AATGGAGACC	TTTCCATGAG AAAGGTACTC	CCGGTGTCCA GGCCACAGGT	CGCTCGGTGA GCGAGCCACT
4401	CGAAAAGGCT GCTTTTCCGA	GTCCGTGTCC CAGGCACAGG	CCGTATACAG GGCATATGTC	ACTTGAGAGG TGAACTCTCC	CCTGTCCTCG GGACAGGAGC
4451	AGCGGTGTTC TCGCCACAAG	CGCGGTCCTÇ GCGCCAGGAG	CTCGTATAGA GAGCATATCT	AACTCGGACC TTGAGCCTGG	ACTCTGAGAC TGAGACTCTG
4501	AAAGGCTCGC TTTCCGAGCG	GTCCAGGCCA CAGGTCCGGT	GCACGAAGGA CGTGCTTCCT	GGCTAAGTGG CCGATTCACC	GAGGGGTAGC CTCCCCATCG
4551	GGTCGTTGTC CCAGCAACAG	CACTAGGGGG GTGATCCCCC	TCCACTCGCT AGGTGAGCGA	CCAGGGTGTG GGTCCCACAC	AAGACACATG TTCTGTGTAC
4601	TCGCCCTCTT AGCGGGAGAA	CGGCATCAAG GCCGTAGTTC	GAAGGTGATŢ CTTCCACTAA	GGTTTGTAGG CCAAACATCC	TGTAGGCCAC ACATCCGGTG

Figure 27E

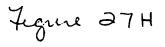
4701					CTGTTGGGGT GACAACCCCA
4751					GATTGTCAGT
	CTCATGAGGG	AGACTTTTCG	CCCGTACTGA	AGACGCGATT	CTAACAGTCA
4801					GTGATGCCTT CACTACGGAA
4851					TTTGTTGTCA AAACAACAGT
4901					ACTTGGCGAT TGAACCGCTA
4951			•		TTGGCCGCGA
		CAAACCAAAA			
5001		CACGTATTCG GTGCATAAGC			
5051		CGTCGGGCAC GCAGCCCGTG			
5101		TCAACGCTGG AGTTGCGACC			
5151		ccccccccc			
5201		CGTCCGGGGG GCAGGCCCCC			
5251		TCGAAGTAGT AGCTTCATCA			
5301		GCGGGCGCA CGCCCGCCGT			
5351		TGGGGTGGGT ACCCCACCCA			
5401	GTAAACGTAG CATTTGCATC	AGGGGCTCTC TCCCCGAGAG			
5451	TTCCACCGCG AAGGTGGCGC	GATGCTGGCG CTACGACCGC			
5501	GCGAGGAGGT CGCTCCTCCA	CGGGACCGAG GCCCTGGCTC			
5551	GACTATCTGC CTGATAGACG	CTGAAGATGG GACTTCTACC			

Figure 27F

5651	GAGGCGTAGG	AGTCGCGCAG	CTTGTTGACC	AGCTCGGCGG	TGACCTGCAC
	CTCCGCATCC	TCAGCGCGTC	GAACAACTGG	TCGAGCCGCC	ACTGGACGTG
5701	GTCTAGGGCG	CAGTAGTCCA	GGGTTTCCTT	GATGATGTCA	TACTTATCCT
	CAGATCCCGC	GTCATCAGGT	CCCAAAGGAA	CTACTACAGT	ATGAATAGGA
5751	GTCCCTTTTT	TTTCCACAGC	TCGCGGTTGA	GGACAAACTC	TTCGCGGTCT
	CAGGGAAAAA	AAAGGTGTCG	AGCGCCAACT	CCTGTTTGAG	AAGCGCCAGA
5801	TTCCAGTACT	CTTGGATCGG	AAACCCGTCG	GCCTCCGAAC	GGTAAGAGCC
	AAGGTCATGA	GAACCTAGCC	TTTGGGCAGC	CGGAGGCTTG	CCATTCTCGG
5851	TAGCATGTAG	AACTGGTTGA	CGGCCTGGTA	GGCGCAGCAT	CCCTTTTCTA
	ATCGTACATC	TTGACCAACT	GCCGGACCAT	CCGCGTCGTA	GGGAAAAGAT
5901	CGGGTAGCGC	GTATGCCTGC	GCGGCCTTCC	GGAGCGAGGT	GTGGGTGAGC
	GCCCATCGCG	CATACGGACG	CGCCGGAAGG	CCTCGCTCCA	CACCCACTCG
5951	GCAAAGGTGT	CCCTGACCAT	GACTTTGAGG	TACTGGTATT	TGAAGTCAGT
	CGTTTCCACA	GGGACTGGTA	CTGAAACTCC	ATGACCATAA	ACTTCAGTCA
6001	GTCGTCGCAT	CCGCCCTGCT	CCCAGAGCAA	AAAGTCCGTG	CGCTTTTTGG
	CAGCAGCGTA	GGCGGGACGA	GGGTCTCGTT	TTTCAGGCAC	GCGAAAAACC
6051	AACGCGGATT	TGGCAGGGCG	AAGGTGACAT	CGTTGAAGAG	TATCTTTCCC
	TTGCGCCTAA	ACCGTCCCGC	TTCCACTGTA	GCAACTTCTC	ATAGAAAGGG
6101	GCGCGAGGCA	TAAAGTTGCG	TGTGATGCGG	AAGGGTCCCG	GCACCTCGGA
	CGCGCTCCGT	ATTTCAACGC	ACACTACGCC	TTCCCAGGGC	CGTGGAGCCT
6151	ACGGTTGTTA	ATTACCTGGG	CGGCGAGCAC	GATCTCGTCA	AAGCCGTTGA
	TGCCAACAAT	TAATGGACCC	GCCGCTCGTG	CTAGAGCAGT	TTCGGCAACT
6201	TGTTGTGGCC	CACAATGTAA	AGTTCCAAGA	AGCGCGGGAT	GCCCTTGATG
	ACAACACCGG	GTGTTACATT	TCAAGGTTCT	TCGCGCCCTA	CGGGAACTAC
6251	GAAGGCAATT	TTTTAAGTTC	CTCGTAGGTG	AGCTCTTCAG	GGGAGCTGAG
	CTTCCGTTAA	AAAATTCAAG	GAGCATCCAC	TCGAGAAGTC	CCCTCGACTC
6301	CCCGTGCTCT	GAAAGGGCCC	AGTCTGCAAG	ATGAGGGTTG	GAAGCGACGA
	GGGCACGAGA	CTTTCCCGGG	TCAGACGTTC	TACTCCCAAC	CTTCGCTGCT
6351	ATGAGCTCCA	CAGGTCACGG	GCCATTAGCA	TTTGCAGGTG	GTCGCGAAAG
	TACTCGAGGT	GTCCAGTGCC	CGGTAATCGT	AAACGTCCAC	CAGCGCTTTC
6401	GTCCTAAACT	GGCGACCTAT	GGCCATTTTT	TCTGGGGTGA	TGCAGTAGAA
	CAGGATTTGA	CCGCTGGATA	CCGGTAAAAA	AGACCCCACT	ACGTCATCTT
6451	GGTAAGCGGG	TCTTGTTCCC	AGCGGTCCCA	TCCAAGGTTC	GCGGCTAGGT
	CCATTCGCCC	AGAACAAGGG	TCGCCAGGGT	AGGTTCCAAG	CGCCGATCCA
6501	CTCGCGCGGC	AGTCACTAGA	GGCTCATCTC	CGCCGAACTT	CATGACCAGC
	GAGCGCGCCG	TCAGTGATCT	CCGAGTAGAG	GCGGCTTGAA	GTACTGGTCG

Figure 276

6601					GAGCCGATCG
6651		GATCTCCCGC CTAGAGGGCG			ATTGATGTGG TAACTACACC
6701		AGTCCCTGCG TCAGGGACGC			
6751		CAGTACTGGC GTCATGACCG			
6801	•	ACGACCGCGC TGCTGGCGCG		-	
6851		GGTTTGGCTG CCAAACCGAC			
6901		TGCTCGAGGG ACGAGCTCCC			
6951		AGTCCAGATG TCAGGTCTAC			
7001		GATGGGAGCT CTACCCTCGA			
7051	-•	AGCTCCTGCA TCGAGGACGT			
7101		CAGGTGATAC GTCCACTATG			
7151		GCAAGAGGCC CGTTCTCCGG			
7201	GCCGCCGCC	TGGGCCGCGG ACCCGGCGCC	CCCACAGGAA	CCTACTACGT	AGATTTTCGC
7251	CACTGCGCCC	CGAGCCCCCG	CTCCATCCCC	CCCGAGGCCT	GGGCGCCCT
	CTCCCCGTC	GGGCACGTCG CCCGTGCAGC	CGCGCGCGC	GCCCGTCCTC	GACCACGACG
	CGCGCATCCA	TGCTGGCGAA ACGACCGCTT	GCGCTGCTGC	GCCGCCAACT	AGAGGACTTA
	GACCGCGGAG	TGCGTGAAGA	GCTGCCCGGG	CCACTCGAAC	TTGGACTTTC
7451		AGAATCAATT TCTTAGTTAA			



7551	CTGCTCGATC	TCTTCCTCCT	GGAGATCTCC	GCGTCCGGCT	CGCTCCACGG
	GACGAGCTAG	AGAAGGAGGA	CCTCTAGAGG	CGCAGGCCGA	GCGAGGTGCC
7601	TGGCGGCGAG	GTCGTTGGAA	ATGCGGGCCA	TGAGCTGCGA	GAAGGCGTTG
	ACCGCCGCTC	CAGCAACCTT	TACGCCCGGT	ACTCGACGCT	CTTCCGCAAC
7651	AGGCCTCCCT	CGTTCCAGAC	GCGGCTGTAG	ACCACGCCCC	CTTCGGCATC
	TCCGGAGGGA	GCAAGGTCTG	CGCCGACATC	TGGTGCGGGG	GAAGCCGTAG
7701	CCCCCCCCC	ATGACCACCT TACTGGTGGA	GCGCGAGATT CGCGCTCTAA	GAGCTCCACG CTCGAGGTGC	TGCCGGGCGA ACGGCCCGCT
7751	AGACGGCGTA	GTTTCGCAGG	CGCTGAAAGA	GGTAGTTGAG	GGTGGTGGCG
	TCTGCCGCAT	CAAAGCGTCC	GCGACTTTCT	CCATCAACTC	CCACCACCGC
7801	GTGTGTTCTG	CCACGAAGAA	GTACATAACC	CAGCGTCGCA	ACGTGGATTC
	CACACAAGAC	GGTGCTTCTT	CATGTATTGG	GTCGCAGCGT	TGCACCTAAG
7851	GTTGATATCC	CCCAAGGCCT	CAAGGCGCTC	CATGGCCTCG	TAGAAGTCCA
	CAACTATAGG	GGGTTCCGGA	GTTCCGCGAG	GTACCGGAGC	ATCTTCAGGT
7901	CGGCGAAGTT	GAAAAACTGG	GAGTTGCGCG	CCGACACGGT	TAACTCCTCC
	GCCGCTTCAA	CTTTTTGACC	CTCAACGCGC	GGCTGTGCCA	ATTGAGGAGG
7951	TCCAGAAGAC	GGATGAGCTC	GGCGACAGTG	TCGCGCACCT	CGCGCTCAAA
	AGGTCTTCTG	CCTACTCGAG	CCGCTGTCAC	AGCGCGTGGA	GCGCGAGTTT
8001	GGCTACAGGG	GCCTCTTCTT	CTTCTTCAAT	CTCCTCTTCC	ATAAGGGCCT
	CCGATGTCCC	CGGAGAAGAA	GAAGAAGTTA	GAGGAGAAGG	TATTCCCGGA
8051	CCCCTTCTTC	TTCTTCTGGC	GGCGGTGGGG	GAGGGGGGAC	ACGGCGGCGA
	GGGGAAGAAG	AAGAAGACCG	CCGCCACCCC	CTCCCCCTG	TGCCGCCGCT
8101	CGACGCCGCA	CCGGGAGGCG	GTCGACAAAG	CGCTCGATCA	TCTCCCGCG
	GCTGCCGCGT	GGCCCTCCGC	CAGCTGTTTC	GCGAGCTAGT	AGAGGGGCGC
8151	GCGACGGCGC CGCTGCCGCG	ATGGTCTCGG TACCAGAGCC	TGACGGCGCG ACTGCCGCGC	GCCGTTCTCG CGGCAAGAGC	CGGGGGCGCA
8201	GTTGGAAGAC CAACCTTCTG	GCCGCCCGTC CGGCGGGCAG	ATGTCCCGGT TACAGGGCCA	TATGGGTTGG ATACCCAACC	CGGGGGGCTG
8251	CCATGCGGCA	GGGATACGGC	GCTAACGATG	CATCTCAACA	ATTGTTGTGT
	GGTACGCCGT	CCCTATGCCG	CGATTGCTAC	GTAGAGTTGT	TAACAACACA
8301	AGGTACTCCG TCCATGAGGC	CCGCCGAGGG	ACCTGAGCGA TGGACTCGCT	GTCCGCATCG CAGGCGTAGC	ACCGGATCGG TGGCCTAGCC
8351	AAAACCTCTC TTTTGGAGAG	GAGAAAGGCG CTCTTTCCGC	TCTAACCAGT AGATTGGTCA	CACAGTCGCA GTGTCAGCGT	AGGTAGGCTG TCCATCCGAC
8401	AGCACCGTGG TCGTGGCACC	CGGGCGGCAG	CCCCCCCCC	TCGGGGTTGT	TTCTGGCGGA AAGACCGCCT

Figure 27I

8501			GCGCAGGCGG CGCGTCCGCC
8551			CTTTGTAGTA GAAACATCAT
8601	••••	 CCGGCACTTC GGCCGTGAAG	 TCCTCTTGTC AGGAGAACAG
8651		 GCTGCGGCGG CGACGCCGCC	 TGGCCGTAGG ACCGGCATCC
8701		 GCGTGTGACC CGCACACTGG	
8751		CAACGCGCTC GTTGCGCGAG	
8801		AAGTCATCCA TTCAGTAGGT	
8851		 AGTGCAGTTG TCACGTCAAC	
8901		AGAGCTCGGT TCTCGAGCCA	
8951		 TCGTTGCAAG AGCAACGTTC	
9001		CGGCTGGCGG GCCGACCGCC	
9051		 GATCTTCCAA CTAGAAGGTT	
9101		 GTGATGCCGG CACTACGGCC	
9151		CCAGATGTTG GGTCTACAAC	
9201		CGGTCAGGCG GCCAGTCCGC	TTGACGCTCT. AACTGCGAGA
9251	AGACCGTGCA TCTGGCACGT	CTGTAAGCGG GACATTCGCC	
9301	GGATAAATTC CCTATTTAAG	CATGGCGGAC GTACCGCCTG	
9351	ATCCGGCCGT TAGGCCGGCA	CCATGCGGTT GGTACGCCAA	

Figure 27J

9451	CCGCGCCGCC	CTGCTGCGCT GACGACGCGA	AGCTTTTTTG TCGAAAAAAC	GCCACTGGCC CGGTGACCGG	GCGCGCAGCG CGCGCGTCGC
9501	TAAGCGGTTA ATTCGCCAAT		CGAAAGCATT GCTTTCGTAA		
9551	CCGGAGGGTT	ATTTTCCAAG	GGTTGAGTCG	CGGGACCCCC	GGTTCGAGTC
	GGCCTCCCAA	TAAAAGGTTC	CCAACTCAGC	GCCCTGGGGG	CCAAGCTCAG
9601	TCGGACCGGC	CGGACTGCGG	CGAACGGGGG	TTTGCCTCCC	CGTCATGCAA
	AGCCTGGCCG	GCCTGACGCC	GCTTGCCCCC	AAACGGAGGG	GCAGTACGTT
9651	GACCCCGCTT	GCAAATTCCT	CCGGAAACAG	GGACGAGCCC	CTTTTTTGCT
	CTGGGGCGAA	CGTTTAAGGA	GGCCTTTGTC	CCTGCTCGGG	GAAAAAACGA
9701	TTTCCCAGAT AAAGGGTCTA	GCATCCGGTG CGTAGGCCAC	CTGCGGCAGA GACGCCGTCT	TGCGCCCCCC	TCCTCAGCAG AGGAGTCGTC
9751	CGGCAAGAGC	AAGAGCAGCG	GCAGACATGC	AGGGCACCCT	CCCCTCCTCC
	GCCGTTCTCG	TTCTCGTCGC	CGTCTGTACG	TCCCGTGGGA	GGGGAGGAGG
9801	TACCGCGTCA	GGAGGGGCGA	CATCCGCGGT	TGACGCGGCA	GCAGATGGTG
	ATGGCGCAGT	CCTCCCGCT	GTAGGCGCCA	ACTGCGCCGT	CGTCTACCAC
9851	ATTACGAACC TAATGCTTGG	CCCGCGCGCG	CGGGCCCGGC	ACTACCTGGA TGATGGACCT	CTTGGAGGAG GAACCTCCTC
9901	GGCGAGGGCC	TGGCGCGGCT	AGGAGCGCCC	TCTCCTGAGC	GGCACCCAAG
	CCGCTCCCGG	ACCGCGCCGA	TCCTCGCGGG	AGAGGACTCG	CCGTGGGTTC
9951	GGTGCAGCTG	AAGCGTGATA	CGCGTGAGGC	GTACGTGCCG	CGGCAGAACC
	CCACGTCGAC	TTCGCACTAT	GCGCACTCCG	CATGCACGGC	GCCGTCTTGG
10001	TGTTTCGCGA	CCGCGAGGGA	GAGGAGCCCG	AGGAGATGCG	GGATCGAAAG
	ACAAAGCGCT	GGCGCTCCCT	CTCCTCGGGC	TCCTCTACGC	CCTAGCTTTC
10051	TTCCACGCAG	GGCGCGAGCT	GCGGCATGGC	CTGAATCGCG	AGCGGTTGCT
	AAGGTGCGTC	CCGCGCTCGA	CGCCGTACCG	GACTTAGCGC	TCGCCAACGA
10101	GCGCGAGGAG CGCGCTCCTC	GACTTTGAGC CTGAAACTCG	CCGACGCGCG	AACCGGGATT TTGGCCCTAA	AGTCCCGCGC TCAGGGCGCG
10151	GCGCACACGT CGCGTGTGCA	CCCCCCCCC	GACCTGGTAA CTGGACCATT	CCGCATACGA GGCGTATGCT	GCAGACGGTG CGTCTGCCAC
10201	AACCAGGAGA	TTAACTTTCA	AAAAAGCTTT	AACAACCACG	TGCGTACGCT
	TTGGTCCTCT	AATTGAAAGT	TTTTTCGAAA	TTGTTGGTGC	ACGCATGCGA
10251	TGTGGCGCGC	GAGGAGGTGG	CTATAGGACT	GATGCATCTG	TGGGACTTTG
	ACACCGCGCG	CTCCTCCACC	GATATCCTGA	CTACGTAGAC	ACCCTGAAAC
10301	TAAGCGCGCT	GGAGCAAAAC	CCAAATAGCA	AGCCGCTCAT	GGCGCAGCTG
	ATTCGCGCGA	CCTCGTTTTG	GGTTTATCGT	TCGGCGAGTA	CCGCGTCGAC

Figure 27K

10401				 TTGATAAACA AACTATTTGT
10451		CATAGTGGTG GTATCACCAC		GGCTGACAAG CCGACTGTTC
10501		TCAACTATTC AGTTGATAAG		TTTACGCCCG AAATGCGGGC
10551		CATACCCCTT GTATGGGGAA	· · · · · · · · · · · · · · · · · · ·	
10601		CATGCGCATG GTACGCGTAC		
10651		ATCGCAACGA TAGCGTTGCT		
10701		CTCAGCGACC GAGTCGCTGG		
10751		GGGCAGCGGC CCCGTCGCCG		
10801		TGCGCTGGGC ACGCGACCCG		
10851		GGGCTGGCGG CCCGACCGCC		
10901		ATATGACGAG TATACTGCTC		
10951		TGATGTTTCT ACTACAAAGA		
11001		CGGCGCTGCA GCCGCGACGT		
11051	• • • • • • • • • • • • • • • • • • • •	CAGGTCATGG GTCCAGTACC		
11101	CTGACGCGTT GACTGCGCAA			
11151	GAAGCGGTGG CTTCGCCACC			
11201	GATCGTAAAC CTAGCATTTG			
11251	GCCTGGTCTA CGGACCAGAT			

Figure 27L

11351	GGCGCAGCGT CCGCGTCGCA	GAGCGCGCGC CTCGCGCGCG	AGCAGCAGGG TCGTCGTCCC	CAACCTGGGC GTTGGACCCG	TCCATGGTTG AGGTACCAAC
11401	CACTAAACGC GTGATTTGCG	CTTCCTGAGT GAAGGACTCA	ACACAGCCCG TGTGTCGGGC	CCAACGTGCC GGTTGCACGG	GCGGGGACAG CGCCCCTGTC
11451	GAGGACTACA CTCCTGATGT	CCAACTTTGT GGTTGAAACA	GAGCGCACTG CTCGCGTGAC	CGGCTAATGG GCCGATTACC	TGACTGAGAC ACTGACTCTG
11501	ACCGCAAAGT TGGCGTTTCA	GAGGTGTACC CTCCACATGG	AGTCTGGGCC TCAĢACCCGG	AGACTATTTT TCTGATAAAA	TTCCAGACCA AAGGTCTGGT
11551	CATCTGTTCC	GGACGTCTGG	CATTTGGACT	GCCAGGCTTT CGGTCCGAAA	GTTTTTGAAC
11601	GTCCCCGACA	CCCCCACGC	CCGAGGGTGT	GGCGACCGCG CCGCTGGCGC	GCTGGCACAG
11.651	ATCGAACGAC	TGCGGGTTGA	GCGCGGACAA	GCTGCTGCTA CGACGACGAT	TATCGCGGGA
11701	AGTGCCTGTC	ACCGTCGCAC	AGGGCCCTGT	CATACCTAGG GTATGGATCC	AGTGAACGAC
11751	TGTGACATGG	CGCTCCGGTA	TCCAGTCCGC	CATGTGGACG GTACACCTGC	TCGTATGAAA
11801	GGTCCTCTAA	TGTTCACAGT	CGGCGCGCGA	GGGGCAGGAG CCCCGTCCTC	CTGTGCCCGT
11851	CGGACCTCCG	TTGGGATTTG	ATGGACGACT	CCAACCGGCG GGTTGGCCGC	CGTCTTCTAG
11901	GGGAGCAACG	TGTCAAATTT	GTCGCTCCTC	GAGCGCATTT CTCGCGTAAA	ACGCGATGCA
11951	CGTCGTCTCG	CACTCGGAAT	TGGACTACGC	CGACGGGGTA GCTGCCCCAT	TGCGGGTCGC
12001	ACCGCGACCT	GTACTGGCGC	GCGTTGTACC	AACCGGGCAT TTGGCCCGTA	CATACGGAGT
	TTGGCCGGCA	AATAGTTGGC	GGATTACCTG	ATGAACGTAG	
	GCACTTGGGG	CTCATAAAGT	GGTTACGGTA	GAACTTGGGC	CACTGGCTAC GTGACCGATG
	GCGGGGGACC	AAAGATGTGG	CCCCCTAAGC	TCCACGGGCT	GGGTAACGAT CCCATTGCTA
12201	GGATTCCTCT CCTAAGGAGA	GGGACGACAT CCCTGCTGTA	AGACGACAGC TCTGCTGTCG	GTGTTTTCCC CACAAAAGGG	CGCAACCGCA GCGTTGGCGT

Figure 27 M

12301	AGGAAAGCTT TCCTTTCGAA			CCGATCTAGG	
12351				AGCTTGATAG	
				TCGAACTATC	
12401				GGGCGAGGAG CCCGCTCCTC	
12451				AAAACCTGCC	•
•				TTTTGGACGG	
12501				AAGATGAGTA TTCTACTCAT	
12551	GTACGCGCAG				
				GGCGCGGGC	
12601				TGTGGGAGGA ACACCCTCCT	
12651				GGGAGTGGCA CCCTCACCGT	
12701	GCACCTTCGC				
22,02			-	AATTTTTTTT	
12751	ATGCAAAATA TACGTTTTAT			GCACCGAGCG CGTGGCTCGC	
12801				ATGTATGAGG	
				TACATACTCC	•
12851				GCCAGTGGCG CGGTCACCGC	
12901				CGTTTGTGCC GCAAACACGG	
.12951				CGTTACTCTG GCAATGAGAC	
13001	CCTATTCGAC				
	GGATAAGCTG	TGGTGGGCAC	ACATGGACCA	CCTGTTGTTC	AGTTGCCTAC
13051	TGGCATCCCT ACCGTAGGGA			GCAACTTTCT CGTTGAAAGA	
13101	ATTCAAAACA TAAGTTTTGT			GCAAGCACAC CGTTCGTGTG	
13151	TCTTGACGAC AGAACTGCTG			CCTGAAAACC GGACTTTTGG	

Figure 27 N

13251	CGGGTGATGG	TGTCGCGCTT	GCCTACTAAG	GACAATCAGG	TGGAGCTGAA
	GCCCACTACC	ACAGCGCGAA	CGGATGATTC	CTGTTAGTCC	ACCTCGACTT
13301	ATACGAGTGG	GTGGAGTTCA	CGCTGCCCGA	GGGCAACTAC	TCCGAGACCA
	TATGCTCACC	CACCTCAAGT	GCGACGGGCT	CCCGTTGATG	AGGCTCTGGT
13351	TGACCATAGA	CCTTATGAAC	AACGCGATCG	TGGAGCACTA	CTTGAAAGTG
	ACTGGTATCT	GGAATACTTG	TTGCGCTAGC	ACCTCGTGAT	GAACTTTCAC
13401	GGCAGACAGA	ACGGGGTTCT	GGAAAGCGAC	ATCGGGGTAA	AGTTTGACAC
	CCGTCTGTCT	TGCCCCAAGA	CCTTTCGCTG	TAGCCCCATT	TCAAACTGTG
13451	CCGCAACTTC	AGACTGGGGT	TTGACCCCGT	CACTGGTCTT	GTCATGCCTG
	GGCGTTGAAG	TCTGACCCCA	AACTGGGGCA	GTGACCAGAA	CAGTACGGAC
13501	GGGTATATAC	AAACGAAGCC	TTCCATCCAG	ACATCATTTT	GCTGCCAGGA
	CCCATATATG	TTTGCTTCGG	AAGGTAGGTC	TGTAGTAAAA	CGACGGTCCT
13551	TGCGGGGTGG	ACTTCACCCA	CAGCCGCCTG	AGCAACTTGT	TGGGCATCCG
	ACGCCCCACC	TGAAGTGGGT	GTCGGCGGAC	TCGTTGAACA	ACCCGTAGGC
13601	CAAGCGGCAA	CCCTTCCAGG	AGGGCTTTAG	GATCACCTAC	GATGATCTGG
	GTTCGCCGTT	GGGAAGGTCC	TCCCGAAATC	CTAGTGGATG	CTACTAGACC
13651	TCCCACCATT	CATTCCCGCA GTAAGGGCGT	GACAACCTAC	ACCTGCGGAT	GGTCCGCTCG
13701	AACTTTCTAC	ACACCGAACA TGTGGCTTGT	CCCGCCCCCA	CCGCGTCCGC	CGTCGTTGTC
13751	GTCACCGTCG	GGCGCGGAAG CCGCGCCTTC	TCTTGAGGTT	GCGCCGTCGG	CGCCGTTACG
13801	TCGGCCACCT	GGACATGAAC CCTGTACTTG	CTAGTACGGT	AAGCGCCGCT	GTGGAAACGG
13851	TGTGCCCGAC	AGGAGAAGCG TCCTCTTCGC	GCGACTCCGG	CTTCGTCGCC	GGCTTCGACG
13901	GCGGGGGCGA	GCGCAACCCG CGCGTTGGGC	TCCAGCTCTT	CGGAGTCTTC	TTTGGCCACT
	AGTTTGGGGA	CTGTCTCCTG	TCGTTCTTTG	CGTCAATGTT	
	TTACTGTCGT	GGAAGTGGGT	CATGGCGTCG	ACCATGGAAC	
	GCCGCTGGGA	GTCTGGCCTT	AGGCGAGTAC	CTGGGACGAA	TGCACTCCTG ACGTGAGGAC
14101	ACGTAACCTG	CGGCTCGGAG	CAGGTCTACT	GGTCGTTGCC	AGACATGATG
	TGCATTGGAC	GCCGAGCCTC	GTCCAGATGA	CCAGCAACGG	TCTGTACTAC

Tigure 270

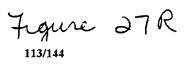
14201					C TACAACGACC
	CCACCCGCG	G CTCGACAAC	g ggcacgtga	G GTTCTCGAA	G ATGTTGCTGG
14251					T GACCCACGTG
	TCCGGCAGA	r Gagggttga	G TAGGCGGTC	A AATGGAGAG	A CTGGGTGCAC
14301	TTCAATCGCT	TTCCCGAGA	A CCAGATTTT	G GCGCGCCGG	CAGCCCCCAC
					G GTCGGGGGTG
14351					CACGGGACGC
					GTGCCCTGCG
14401					CATTACTGAC
			•		GTAATGACTG
14451	GCCAGACGCC	GCACCTGCCC	CTACGTTTAC	AAGGCCCTGG	GCATAGTCTC
		•			CGTATCAGAG
14501					TCCATCCTTA
14551					AGGTAGGAAT
14551					AAGCAAGATG
					TTCGTTCTAC
14601					GCGTGCGCGG
14651					CGCACGCGCC
14651					ACTGGGCGCA TGACCCGCGT
14701					
14701				TGGAGGAGGC ACCTCCTCCG	
14751					
74/21				GACGCGGCCA CTGCGCCGGT	
14001					
14801				GAAGAGACGG CTTCTCTGCC	
	concococc 1	COGGCCGCGA	IACGAIIIIA	CITCICIGCC	GCCTCCGCGC
14851	TAGCACGTCG	CCACCGCCGC	CGACCCGGCA	CTGCCGCCCA	ACGCGCGGCG
	ATCGTGCAGC	GGTGGCGGCG	GCTGGGCCGT	GACGGCGGGT	TGCGCGCCGC
14901	GCGGCCCTGC	TTAACCGCGC	ACGTCGCACC	GGCCGACGGG	CGGCCATGCG
			•	CCGGCTGCCC	
14951	GGCCGCTCGA				
	CCGGCGAGCT				
15001	GGCGACGAGC	GGCCGCCGCA	GCAGCCGCGG	CCATTAGTGC	TATGACTCAG
	CCGCTGCTCG				
15051	GGTCGCAGGG				
	CCAGCGTCCC	CGTTGCACAT	AACCCACGCG	CTGAGCCAAT	CGCCGGACGC



15151	ACTTAGACTC	GTACTGTTGT	ATGTATCCAG	GCCGCCGCCG	GCGCAACGAA
	TGAATCTGAG	CATGACAACA	TACATAGGTC	CGGCGGCGGC	CGCGTTGCTT
15201	GCTATGTCCA	AGCGCAAAAT	CAAAGAAGAG	ATGCTCCAGG	TCATCGCGCC
	CGATACAGGT	TCGCGTTTTA	GTTTCTTCTC	TACGAGGTCC	AGTAGCGCGG
15251	GGAGATCTAT	GGCCCCCGA	AGAAGGAAGA	GCAGGATTAC	AAGCCCCGAA
	CCTCTAGATA	CCGGGGGCT	TCTTCCTTCT	CGTCCTAATG	TTCGGGGCTT
15301	AGCTAAAGCG	GGTCAAAAAG	AAAAAGAAAG	ATGATGATGA	TGAACTTGAC
	TCGATTTCGC	CCAGTTTTTC	TTTTTCTTTC	TACTACTACT	ACTTGAACTG
15351	GACGAGGTGG	AACTGCTGCA	CGCTACCGCG	CCCAGGCGAC	GGGTACAGTG
	CTGCTCCACC	TTGACGACGT	GCGATGGCGC	GGGTCCGCTG	CCCATGTCAC
15401	GAAAGGTCGA	CGCGTAAAAC	GTGTTTTGCG	ACCCGGCACC	ACCGTAGTCT
	CTTTCCAGCT	GCGCATTTTG	CACAAAACGC	TGGGCCGTGG	TGGCATCAGA
15451	TTACGCCCGG	TGAGCGCTCC	ACCCGCACCT	ACAAGCGCGT	GTATGATGAG
	AATGCGGGCC	ACTCGCGAGG	TGGGCGTGGA	TGTTCGCGCA	CATACTACTC
15501	GTGTACGGCG	ACGAGGACCT	GCTTGAGCAG	GCCAACGAGC	GCCTCGGGGA
	CACATGCCGC	TGCTCCTGGA	CGAACTCGTC	CGGTTGCTCG	CGGAGCCCCT
15551	GTTTGCCTAC	GGAAAGCGGC	ATAAGGACAT	GCTGGCGTTG	CCGCTGGACG
	CAAACGGATG	CCTTTCGCCG	TATTCCTGTA	CGACCGCAAC	GGCGACCTGC
15601	AGGGCAACCC	AACACCTAGC	CTAAAGCCCG	TAACACTGCA	GCAGGTGCTG
	TCCCGTTGGG	TTGTGGATCG	GATTTCGGGC	ATTGTGACGT	CGTCCACGAC
15651	CCCGCGCTTG	CACCGTCCGA	AGAAAAGCGC	GGCCTAAAGC	GCGAGTCTGG
	GGGCGCGAAC	GTGGCAGGCT	TCTTTTCGCG	CCGGATTTCG	CGCTCAGACC
15701	TGACTTGGCA	CCCACCGTGC	AGCTGATGGT	ACCCAAGCGC	CAGCGACTGG
	ACTGAACCGT	GGGTGGCACG	TCGACTACCA	TGGGTTCGCG	GTCGCTGACC
15751	AAGATGTCTT	GGAAAAAATG	ACCGTGGAAC	CTGGGCTGGA	GCCCGAGGTC
	TTCTACAGAA	CCTTTTTTAC	TGGCACCTTG	GACCCGACCT	CGGGCTCCAG
15801	CGCGTGCGGC GCGCACGCCG	CAATCAAGCA GTTAGTTCGT	GGTGGCGCCG	GGACTGGGCG CCTGACCCGC	TGCAGACCGT ACGTCTGGCA
15851	GGACGTTCAG	ATACCCACTA	CCAGTAGCAC	CAGTATTGCC	ACCGCCACAG
	CCTGCAAGTC	TATGGGTGAT	GGTCATCGTG	GTCATAACGG	TGGCGGTGTC
15901	AGGGCATGGA	GACACAAACG	TCCCCGGTTG	CCTCAGCGGT	GGCGGATGCC
	TCCCGTACCT	CTGTGTTTGC	AGGGGCCAAC	GGAGTCGCCA	CCGCCTACGG
15951	GCGCTGCAGG CGCCACGTCC	CGGTCGCTGC GCCAGCGACG	GGCCGCGTCC	AAGACCTCTA TTCTGGAGAT	CGGAGGTGCA GCCTCCACGT
16001	AACGGACCCG TTGCCTGGGC	TGGATGTTTC ACCTACAAAG	GCGTTTCAGC CGCAAAGTCG	CCCCCGGCGC	CCGCGCCGTT

Figure 270

16051		CCCCCCCCC		
16101		CGCCTACCCC GCGGATGGGG		
16151		ACTACCCGAC TGATGGGCTG		
16201		CCAGCCCGTG GGTCGGGCAC		
16251		GCAGGACCCT CGTCCTGGGA		
16301		AAGCCGGTCT TTCGGCCAGA		
16351		TTTCCCGGTG AAAGGGCCAC		
16401		CCGGCCACGG GGCCGGTGCC		
16451	- -	CGCGCGTCGC GCGCGCAGCG		_
16501		ACTGATCGCC TGACTAGCGG	•	
16551		TGCAGGCGCA ACGTCCGCGT		
16601		AATAAAAAGT TTATTTTTCA		
16651		GAATGGAAGA CTTACCTTCT		
16701	CGGCTCGCGC GCCGAGCGCG	CCGTTCATGG GGCAAGTACC		
16751	TGAGCGGTGG ACTCGCCACC	CGCCTTCAGC GCGGAAGTCG		
16801	TTCGGTTCCA AAGCCAAGGT	CCGTTAAGAA GGCAATTCTT		
16851	AGGCCAGATG TCCGGTCTAC	CTGAGGGATA GACTCCCTAT		
16901	TGGTAGATGG ACCATCTACC	CCTGGCCTCT GGACCGGAGA		
16951	CAGGCAGTGC GTCCGTCACG	AAAATAAGAT TTTTATTCTA		



17051	AAAAGCGTCC	GCGCCCCGAC	AGGGAAGAAA	CTCTGGTGAC	GCAAATAGAC
	TTTTCGCAGG	CGCGGGGCTG	TCCCTTCTTT	GAGACCACTG	CGTTTATCTG
17161	GAGCCTCCCT	CGTACGAGGA	GGCACTAAAG	CAAGGCCTGC	CCACCACCCG
	CTCGGAGGGA	GCATGCTCCT	CCGTGATTTC	GTTCCGGACG	GGTGGTGGGC
17151	TCCCATCGCG	CCCATGGCTA	CCGGAGTGCT	GGGCCAGCAC	ACACCCGTAA
	AGGGTAGCGC	GGGTACCGAT	GGCCTCACGA	CCCGGTCGTG	TGTGGGCATT
17201	CGCTGGACCT	GCCTCCCCCC	GCCGACACCC	AGCAGAAACC	TGTGCTGCCA
	GCGACCTGGA	CGGAGGGGGG	CGGCTGTGGG	TCGTCTTTGG	ACACGACGGT
17251	GCCCGACCG	CCGTTGTTGT	AACCCGTCCT	AGCCGCGCGT	CCCTGCGCCG
	CCGGGCTGGC	GGCAACAACA	TTGGGCAGGA	TCGGCGCGCA	GGGACGCGGC
17301	CGCCGCCAGC	GGTCCGCGAT	CGTTGCGGCC	CGTAGCCAGT	GGCAACTGGC
	GCGGCGGTCG	CCAGGCGCTA	GCAACGCCGG	GCATCGGTCA	CCGTTGACCG
17351	AAAGCACACT	GAACAGCATC	GTGGGTCTGG	GGGTGCAATC	CCTGAAGCGC
	TTTCGTGTGA	CTTGTCGTAG	CACCCAGACC	CCCACGTTAG	GGACTTCGCG
17401	CGACGATGCT	TCTGATAGCT	AACGTGTCGT	ATGTGTGTCA	TGTATGCGTC
	GCTGCTACGA	AGACTATCGA	TTGCACAGCA	TACACACAGT	ACATACGCAG
17451	CATGTCGCCG GTACAGCGGC	CCAGAGGAGC GGTCTCCTCG	TGCTGAGCCG ACGACTCGGC	CCGCGCGCCCC	GCTTTCCAAG CGAAAGGTTC
17501	ATGGCTACCC	CTTCGATGAT	GCCGCAGTGG	TCTTACATGC	ACATCTCGGG
	TACCGATGGG	GAAGCTACTA	CGGCGTCACC	AGAATGTACG	TGTAGAGCCC
17551	CCAGGACGCC	TCGGAGTACC	TGAGCCCCGG	GCTGGTGCAG	TTTGCCCGCG
	GGTCCTGCGG	AGCCTCATGG	ACTCGGGGCC	CGACCACGTC	AAACGGGCGC
17601	CCACCGAGAC	GTACTTCAGC	CTGAATAACA	AGTTTAGAAA	CCCCACGGTG
	GGTGGCTCTG	CATGAAGTCG	GACTTATTGT	TCAAATCTTT	GGGGTGCCAC
17651	GCGCCTACGC	ACGACGTGAC	CACAGACCGG	TCCCAGCGTT	TGACGCTGCG
	CGCGGATGCG	TGCTGCACTG	GTGTCTGGCC	AGGGTCGCAA	ACTGCGACGC
17701	GTTCATCCCT	GTGGACCGTG	AGGATACTGC	GTACTCGTAC	AAGGCGCGGT
	CAAGTAGGGA	CACCTGGCAC	TCCTATGACG	CATGAGCATG	TTCCGCGCCA
17751	TCACCCTAGC	TGTGGGTGAT	AACCGTGTGC	TGGACATGGC	TTCCACGTAC
	AGTGGGATCG	ACACCCACTA	TTGGCACACG	ACCTGTACCG	AAGGTGCATG
17801	TTTGACATCC	GCGGCGTGCT	GGACAGGGGC	CCTACTTTTA	AGCCCTACTC
	AAACTGTAGG	CGCCGCACGA	CCTGTCCCCG	GGATGAAAAT	TCGGGATGAG
17851	TGGCACTGCC	TACAACGCCC	TGGCTCCCAA	GGGTGCCCCA	AATCCTTGCG
	ACCGTGACGG	ATGTTGCGGG	ACCGAGGGTT	CCCACGGGGT	TTAGGAACGC
17901	AATGGGATGA	AGCTGCTACT	GCTCTTGAAA	TAAACCTAGA	AGAAGAGGAC
	TTACCCTACT	TCGACGATGA	CGAGAACTTT	ATTTGGATCT	TCTTCTCCTG

Figure 275

17951					AAAAAACTCA TTTTTTGAGT
18001		CAGGCGCCTT GTCCGCGGAA			AAGGAGGGTA TTCCTCCCAT
18051		TGTCGAAGGT ACAGCTTCCA			TAAAACATTT ATTTTGTAAA
18101		CTCAAATAGG GAGTTTATCC			
18151		GGGAGAGTCC CCCTCTCAGG		-	
18201		TGCAAAACCC ACGTTTTGGG			
18251		AAAATGGAAA TTTTACCTTT			
18301	•	GAGGCAGCCG CTCCGTCGGC	•		
18351		CAGTGAAGAT GTCACTTCTA			
18401	TCTTACATGC AGAATGTACG	CCACTATTAA GGTGATAATT			
18451		CCCAACAGGC GGGTTGTCCG			
18501		GTATTACAAC CATAATGTTG			
18551		AGTTGAATGC TCAACTTACG			
18601		CAGCTTTTGC GTCGAAAACG		•	
18651	TTTCTATGTG AAAGATACAC				
18701	ATTGAAAATC TAACTTTTAG				
18751	GGGAGGTGTG CCCTCCACAC				
18801	GTCAGGAAAA CAGTCCTTTT				
18851	GAAATAAGAG CTTTATTCTC				

Figure 27T

18951	AGCTAAAGTA TCGATTTCAT	CAGTCCTTCC GTCAGGAAGG	AACGTAAAAA TTGCATTTTT	TTTCTGATAA AAAGACTATT	CCCAAACACC GGGTTTGTGG
19001		TGAACAAGCG ACTTGTTCGC			
19051	CATTAACCTT GTAATTGGAA	GGAGCACGCT CCTCGTGCGA	GGTCCCTTGA CCAGGGAACT	CTATATGGAC GATATACCTG	AACGTCAACC TTGCAGTTGG
19101	GTAAATTGGT	CCACCGCAAT GGTGGCGTTA	CGACCGGACG	CGATGGCGAG	TTACAACGAC
19151	CCGTTACCAG	GCTATGTGCC CGATACACGG	GAAGGTGTAG	GTCCACGGAG	TCTTCAAGAA
19201	ACGGTAATTT	AACCTCCTTC TTGGAGGAAG	AGGACGGCCC	GAGTATGTGG	ATGCTCACCT
19251	TGAAGTCCTT	GGATGTTAAC CCTACAATTG	TACCAAGACG	TCTCGAGGGA	TCCTTTACTG
19301	GATTCCCAAC	ACGGAGCCAG TGCCTCGGTC	GTAATTCAAA	CTATCGTAAA	CGGAAATGCG
19351	GTGGAAGAAG	CCCATGGCCC GGGTACCGGG	TGTTGTGGCG	GAGGTGCGAA	CTCCGGTACG
19401	AATCTTTGCT	CACCAACGAC GTGGTTGCTG	GTCAGGAAAT	TGCTGATAGA	GAGGCGGCGG
19451	TTGTACGAGA	ACCCTATACC TGGGATATGG	GCGGTTGCGA	TGGTTGCACG	GGTATAGGTA
19501	GGGGAGGCG	AACTGGGCGG TTGACCCGCC	GAAAGGCGCC	GACCCGGAAG	TGCGCGGAAT
19551	TCTGATTCCT	AACCCCATCA TTGGGGTAGT	GACCCGAGCC	CGATGCTGGG	AATAATGTGG
19601	ATGAGACCGA	CTATACCCTA GATATGGGAT	GGATCTACCT	TGGAAAATGG	AGTTGGTGTG
		CACCGGTAAT	GGAAACTGAG	AAGACAGTCG	ACCGGACCGT
		CGAATGGGGG	TTGCTCAAAC	TTTAATTCGC	GAGTCAACTG
		TGTTGCAACG	GGTCACATTG	TACTGGTTTC	TGACCAAGGA
19801	GGTACAAATG CCATGTTTAC	CTAGCTAACT GATCGATTGA	ATAACATTGG TATTGTAACC	CTACCAGGGC GATGGTCCCG	AAGATATAGG

Figure 274

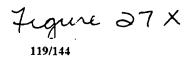
19851				CTTCCAGCCC GAAGGTCGGG
19901				ACCAACAGGT TGGTTGTCCA
19951				TACCTTGCCC ATGGAACGGG
20001				CTATCCGCTT GATAGGCGAA
20051			 	TTCTTTGCGA AAGAAACGCT
20101				TCCATGGGCG AGGTACCCGC
20151				CGCCCACGCG GCGGGTGCGC
20201			 	CCCTTCTTTA GGGAAGAAAT
20251				CCGCACCGCGGGGGGGGGGGGGGGGGGGGGGGGGGGGG
20301		AACCGTGTAC TTGGCACATG	 	
20351		GAAGCAAGCA CTTCGTTCGT	 	
20401		AACTGAAAGC TTGACTTTCG		
20451		ACCTATGACA TGGATACTGT		
20501		CGCCATAGTC GCGGTATCAG		TGGGGGCGTA ACCCCCGCAT
20551	CACTGGATGG GTGACCTACC		 -	GCTACCTCTT CGATGGAGAA
20601	TGAGCCCTTT ACTCGGGAAA			
20651	AGTACGAGTC TCATGCTCAG		 	
	TGTATAACGC ACATATTGCG			
20751	CGCCTGTGGA GCGGACACCT			

Figure 27 V.

20851	CCCAACTCCA	TGCTCAACAG	TCCCCAGGTA	CAGCCCACCC	TGCGTCGCAA
20031	GGGTTGAGGT	ACGAGTTGTC	AGGGGTCCAT	GTCGGGTGGG	ACGCAGCGTT
20901	CCAGGAACAG	CTCTACAGCT	TCCTGGAGCG	CCACTCGCCC	TACTTCCGCA
20302	GGTCCTTGTC	GAGATGTCGA	AGGACCTCGC	GGTGAGCGGG	ATGAAGGCGT
20951	GCCACAGTGC	GCAGATTAGG	AGCGCCACTT	CTTTTTGTCA	CTTGAAAAAC
	CGGTGTCACG	CGTCTAATCC	TCGCGGTGAA	GAAAAACAGT	GAACTTTTTG
21001	ATGTAAAAAT	AATGTACTAG	AGACACTTTC	AATAAAGGCA	AATGCTTTTA
				TTATTTCCGT	
21051	TTTGTACACT	CTCGGGTGAT	TATTTACCCC	CACCCTTGCC	GTCTGCGCCG
				GTGGGAACGG	
21101	TTTAAAAATC	AAAGGGGTTC	TGCCGCGCAT	CGCTATGCGC	CACTGGCAGG
				GCGATACGCG	
21151	GACACGTTGC	GATACTGGTG	TTTAGTGCTC	CACTTAAACT	CAGGCACAAC
				GTGAATTTGA	
21201	CATCCGCGGC	AGCTCGGTGA	AGTTTTCACT	CCACAGGCTG	CGCACCATCA
				GGTGTCCGAC	
21251	CCAACGCGTT	TAGCAGGTCG	GGCGCCGATA	TCTTGAAGTC	GCAGTTGGGG
				AGAACTTCAG	
21301	CCTCCGCCCT	GCGCGCGCGA	GTTGCGATAC	ACAGGGTTGC	AGCACTGGAA
				TGTCCCAACG	
21351	CACTATCAGC	GCCGGGTGGT	GCACGCTGGC	CAGCACGCTC	TTGTCGGAGA
				GTCGTGCGAG	
21401	TCAGATCCGC	GTCCAGGTCC	TCCGCGTTGC	TCAGGGCGAA	CGGAGTCAAC
					GCCTCAGTTG
21451	TTTGGTAGCT	GCCTTCCCAA	AAAGGGCGCG	TGCCCAGGCT	TTGAGTTGCA
			•		AACTCAACGT
21501	CTCGCACCGT	AGTGGCATCA	AAAGGTGACC	GTGCCCGGTC	TGGGCGTTAG
					ACCCGCAATC
21551	GATACAGCGC	CTGCATAAAA	GCCTTGATCT	GCTTAAAAGC	CACCTGAGCC
					GTGGACTCGG
21601	TTTGCGCCTT	CAGAGAAGAA	CATGCCGCAA	GACTTGCCGG	AAAACTGATT
					TTTTGACTAA
21651	GGCCGGACAG	GCCGCGTCGT	GCACGCAGCA	CCTTGCGTCG	GTGTTGGAGA
		•			CACAACCTCT
21701	TCTGCACCAC	ATTTCGGCCC	CACCGGTTCT	TCACGATCTI	GGCCTTGCTA
	AGACGTGGTG	TAAAGCCGGG	GTGGCCAAGA	AGTGCTAGAA	CCGGAACGAT

7. gure 27 W

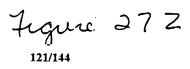
21801					A CACTTAAGCT
	TTAGTGCAC	G AGGAATAAA	T AGTATTACG	A AGGCACATC	T GTGAATTCGA
21851					A GCCCGTGGGC
	GCGGAAGCT	A GAGTCGCGT	C GCCACGTCG	G TGTTGCGCG	r cgggcacccg
21001	D00001000	T			_
21901	ACCACMACC	r TGTAGGTCA	CTCTGCAAA	C GACTGCAGG	T ACGCCTGCAG
	AGCAC TACGA	A ACATECAGT	S GAGACGTTT	G CTGACGTCC	TGCGGACGTC
21951	GAATCGCCCC	ב אתראתרכתר:	י ראאאככיירייי	T CTTCCTCCTC	AAGGTCAGCT
					TTCCAGTCGA
				0.2.0000/.0	. IICCAGICGA
22001	GCAACCCGC	GTGCTCCTCG	TTCAGCCAG	G TCTTGCATAC	GGCCGCCAGA
					CCGGCGGTCT
22051	GCTTCCACTI	GGTCAGGCAG	TAGTTTGAA	TTCGCCTTTA	GATCGTTATC
	CGAAGGTGAA	CCAGTCCGTC	: ATCAAACTT(AAGCGGAAAT	CTAGCAATAG
	0.000000				
22101	CACGTGGTAC	TTGTCCATCA	GCGCGCGCGCG	AGCCTCCATG	CCCTTCTCCC
	GIGCACCAIG	MACAGGTAGT	CGCGCGCGC	TCGGAGGTAC	GGGAAGAGGG
22151	ACCCAGACAC	GATCGCCACA	רשראכרככס	TCATCACCGT	A A COMPONE A COMPON
				A AGTAGTGGCA	
			00100000	· normoreeca	11000001000
22201	TCCGCTTCGC	TGGGCTCTTC	CTCTTCCTCT	TGCGTCCGCA	TACCACGCGC
				ACGCAGGCGT	
22251				TGTGCGCTTA	
	GTGACCCAGC	AGAAGTAAGT	CGGCGGCGTG	ACACGCGAAT	GGAGGAAACG
22301				AACCCACCAT	
	GTACGAACTA	ATCGTGGCCA	CCCAACGACT	TTGGGTGGTA	AACATCGCGG
22351	acarcrrcrc	ずずずぐずずぐぐずぐ	CCTCTCC ACC	ATTACCTCTG	CTCATCCCCC
22334	TGTAGAAGAG	AAAGAAGGAG	CGACAGGTGC	TAATGGAGAC	CACTACCCCC
	1011.0.010.10		co.c	1M1GGAGAC	CACTACCGCC
22401	GCGCTCGGGC	TTGGGAGAAG	GGCGCTTCTT	TTTCTTCTTG	GGCGCAATGG
				AAAGAAGAAC	
22451	CCAAATCCGC	CGCCGAGGTC	GATGGCCGCG	GGCTGGGTGT	GCGCGGCACC
	GGTTTAGGCG	GCGGCTCCAG	CTACCGGCGC	CCGACCCACA	CGCGCCGTGG
22501				TCGGACTCGA	
	TUGUGUAGAA	CACTACTCAG	AAGGAGCAGG	AGCCTGAGCT	ATGCGGCGGA
22551	Carcccrrr	TTTCCCCCCC	CCCCCCAACC	CGGCGGCGAC	000010000
				GCCGCCGCTG	
	CINGUUGAAA			200000000000000000000000000000000000000	
22601	ACGACACGTC	CTCCATGGTT	GGGGGACGTC	GCGCCGCACC	GCGTCCGC
				CGCGGCGTGG	
22651				CGACTGGCCA	
	AGCCCCACC	AAAGCGCGAC	GAGGAGAAGG	GCTGACCGGT	AAAGGAAGAG



22751	CCGCCCCTC	TGAGTTCGCC	ACCACCGCCT	CCACCGATGC	CGCCAACGCG
	GGCGGGGGAG	ACTCAAGCGG	TGGTGGCGGA	GGTGGCTACG	GCGGTTGCGC
22801	CCTACCACCT	TCCCCGTCGA	GGCACCCCCG	CTTGAGGAGG	AGGAAGTGAT
	GGATGGTGGA	AGGGGCAGCT	CCGTGGGGGC	GAACTCCTCC	TCCTTCACTA
22851	TATCGAGCAG	GACCCAGGTT	TTGTAAGCGA	AGACGACGAG	GACCGCTCAG
	ATAGCTCGTC	CTGGGTCCAA	AACATTCGCT	TCTGCTGCTC	CTGGCGAGTC
22901	TACCAACAGA	GGATAAAAAG	CAAGACCAGG	ACAACGCAGA	GGCAAACGAG
	ATGGTTGTCT	CCTATTTTTC	GTTCTGGTCC	TGTTGCGTCT	CCGTTTGCTC
22951	GAACAAGTCG	GGCGGGGGA	CGAAAGGCAT	GGCGACTACC	TAGATGTGGG
	CTTGTTCAGC	CCGCCCCCT	GCTTTCCGTA	CCGCTGATGG	ATCTACACCC
23001	TCTGCTGCAC	GACAACTTCG	TAGACGTCGC		TAATAGACGC
23051	ACGCGTTGCA	AGAGCGCAGC	GATGTGCCCC	TCGCCATAGC	GGATGTCAGC
	TGCGCAACGT	TCTCGCGTCG	CTACACGGGG	AGCGGTATCG	CCTACAGTCG
23101	CTTGCCTACG	AACGCCACCT	ATTCTCACCG	CGCGTACCCC	CCAAACGCCA
	GAACGGATGC	TTGCGGTGGA	TAAGAGTGGC	GCGCATGGGG	GGTTTGCGGT
23151	AGAAAACGGC	ACATGCGAGC	CCAACCCGCG	CCTCAACTTC	TACCCCGTAT
	TCTTTTGCCG	TGTACGCTCG	GGTTGGGCGC	GGAGTTGAAG	ATGGGGCATA
23201	TTGCCGTGCC	AGAGGTGCTT	GCCACCTATC	ACATCTTTTT	CCAAAACTGC
	AACGGCACGG	TCTCCACGAA	CGGTGGATAG	TGTAGAAAAA	GGTTTTGACG
23251	AAGATACCCC	TATCCTGCCG	TGCCAACCGC	AGCCGAGCGG	ACAAGCAGCT
	TTCTATGGGG	ATAGGACGGC	ACGGTTGGCG	TCGGCTCGCC	TGTTCGTCGA
23301	GGCCTTGCGG	CAGGGCGCTG	TCATACCTGA	TATCGCCTCG	CTCAACGAAG
	CCGGAACGCC	GTCCCGCGAC	AGTATGGACT	ATAGCGGAGC	GAGTTGCTTC
23351	TGCCAAAAAT	CTTTGAGGGT	CTTGGACGCG	ACGAGAAGCG	CGCGGCAAAC
	ACGGTTTTTA	GAAACTCCCA	GAACCTGCGC	TGCTCTTCGC	GCGCCGTTTG
23401	GCTCTGCAAC	AGGAAAACAG	CGAAAATGAA	AGTCACTCTG	GAGTGTTGGT
	CGAGACGTTG	TCCTTTTGTC	GCTTTTACTT	TCAGTGAGAC	CTCACAACCA
23451	GGAACTCGAG	GGTGACAACG	CGCGCCTAGC	CGTACTAAAA	CGCAGCATCG
	CCTTGAGCTC	CCACTGTTGC	GCGCGGATCG	GCATGATTTT	GCGTCGTAGC
23501	AGGTCACCCA	CTTTGCCTAC	CCGGCACTTA	ACCTACCCCC	CAAGGTCATG
	TCCAGTGGGT	GAAACGGATG	GGCCGTGAAT	TGGATGGGGG	GTTCCAGTAC
23551	AGCACAGTCA	TGAGTGAGCT	GATCGTGCGC	CGTGCGCAGC	CCCTGGAGAG
	TCGTGTCAGT	ACTCACTCGA	CTAGCACGCG	GCACGCGTCG	GGGACCTCTC
23601	GGATGCAAAT	TTGCAAGAAC	AAACAGAGGA	GGGCCTACCC	GCAGTTGGCG
	CCTACGTTTA	AACGTTCTTG	TTTGTCTCCT	CCCGGATGGG	CGTCAACCGC

Figure 27 Y

23701	GAGCGACGCA	AACTAATGAT	GGCCGCAGTG	CTCGTTACCG	TGGAGCTTGA
	CTCGCTGCGT	TTGATTACTA	CCGGCGTCAC	GAGCAATGGC	ACCTCGAACT
23751	GTGCATGCAG	CGGTTCTTTG	CTGACCCGGA	GATGCAGCGC	AAGCTAGAGG
	CACGTACGTC	GCCAAGAAAC	GACTGGGCCT	CTACGTCGCG	TTCGATCTCC
23801	·			ACGTACGCCA	
	TTTGTAACGT	GATGTGGAAA	GCTGTCCCGA	TGCATGCGGT	CCGGACGTTC
23851	ATCTCCAACG	TGGAGCTCTG	CAACCTGGTC	TCCTACCTTG	GAATTTTGCA
•	TAGAGGTTGC	ACCTCGAGAC	GTTGGACCAG	AGGATGGAAC	CTTAAAACGT
23901	CGAAAACCGC	CTTGGGCAAA	ACGTGCTTCA	TTCCACGCTC	AAGGCCGAGG
	GCTTTTGGCG	GAACCCGTTT	TGCACGAAGT	AAGGTGCGAG	TTCCCGCTCC
23951	CGCGCCGCGA	CTACGTCCGC	GACTGCGTTT	ACTTATTTCT	ATGCTACACC
	GCGCGGCGCT	GATGCAGGCG	CTGACGCAAA	TGAATAAAGA	TACGATGTGG
24001	TGGCAGACGG	CCATGGGCGT	TTGGCAGCAG	TGCTTGGAGG	AGTGCAACCT
	ACCGTCTGCC	GGTACCCGCA	AACCGTCGTC	ACGAACCTCC	TCACGTTGGA
24051	CAAGGAGCTG	CAGAAACTGC	TAAAGCAAAA	CTTGAAGGAC	CTATGGACGG
	GTTCCTCGAC	GTCTTTGACG	ATTTCGTTTT	GAACTTCCTG	GATACCTGCC
24101	CCTTCAACGA	GCGCTCCGTG	GCCGCGCACC	TGGCGGACAT	CATTTTCCCC
	GGAAGTTGCT	CGCGAGGCAC	CGGCGCGTGG	ACCGCCTGTA	GTAAAAGGGG
24151	GAACGCCTGC	TTAAAACCCT	GCAACAGGGT	CTGCCAGACT	TCACCAGTCA
	CTTGCGGACG	AATTTTGGGA	CGTTGTCCCA	GACGGTCTGA	AGTGGTCAGT
24201	AAGCATGTTG	CAGAACTTTA	GGAACTTTAT	CCTAGAGCGC	TCAGGAATCT
	TTCGTACAAC	GTCTTGAAAT	CCTTGAAATA	GGATCTCGCG	AGTCCTTAGA
24251	TGCCCGCCAC	CTGCTGTGCA	CTTCCTAGCG	ACTTTGTGCC	CATTAAGTAC
	ACGGGCGGTG	GACGACACGT	GAAGGATCGC	TGAAACACGG	GTAATTCATG
24301	CGCGAATGCC	CTCCGCCGCT	TTGGGGCCAC	TGCTACCTTC	TGCAGCTAGC
	GCGCTTACGG	GAGGCGGCGA	AACCCCGGTG	ACGATGGAAG	ACGTCGATCG
24351			•	GGAAGACGTG	
	GTTGATGGAA	CGGATGGTGA	GACTGTATTA	CCTTCTGCAC	TCGCCACTGC
24401	GTCTACTGGA				
	CAGATGACCT	CACAGTGACA	GCGACGTTGG	ATACGTGGGG	CGTGGCGAGG
24451	CTGGTTTGCA	ATTCGCAGCT	GCTTAACGAA	AGTCAAATTA	TCGGTACCTT
				TCAGTTTAAT	
24501	TGAGCTGCAG				
	ACTCGACGTC	CCAGGGAGCG	GACTGCTTTT	CAGGCGCCGA	GGCCCCAACT
24551	AACTCACTCC				
	TTGAGTGAGG	CCCCGACACC	TGCAGCCGAA	TGGAAGCGTT	TAAACATGGA



24601	GAGGACTACC CTCCTGATGG	ACGCCCACGA TGCGGGTGCT			
24651		GAGCTTACCG CTCGAATGGC			
24701	GCCAATTGCA CGGTTAACGT	AGCCATCAAC TCGGTAGTTG			
24751		TTTACTTGGA AAATGAACCT			
24801		CCGCAGCCCT GGCGTCGGGA			
24851	AGGATGGCAC TCCTACCGTG	CCAAAAAGAA GGTTTTTCTT			
24901		TGGGACAGTC ACCCTGTCAG			
24951	AGGACATGAT TCCTGTACTA	GGAAGACTGG CCTTCTGACC	GAGAGCCTAG CTCTCGGATC	ACGAGGAAGC TGCTCCTTCG	TTCCGAGGTC AAGGCTCCAG
25001		CAGACGAAAC GTCTGCTTTG			
25051		AAATCGGCAA TTTAGCCGTT			
25101		GCCGGCACTG CGGCCGTGAC			
25151		CCAGGGCCGG GGTCCCGGCC			
25201		CAGCGCCAAG GTCGCGGTTC			
25251	CCATAGTTGC GGTATCAACG	TTGCTTGCAA AACGAACGTT	GACTGTGGGG CTGACACCCC	GCAACATCTC CGTTGTAGAG	CTTCGCCCGC GAAGCGGGCG
25301	CGCTTTCTTC GCGAAAGAAG	TCTACCATCA AGATGGTAGT	CGGCGTGGCC GCCGCACCGG	TTCCCCCGTA AAGGGGGCAT	ACATCCTGCA TGTAGGACGT
25351	TTACTACCGT AATGATGGCA	CATCTCTACA GTAGAGATGT	GCCCATACTG CGGGTATGAC	CACCGCCGCCG	AGCGGCAGCA TCGCCGTCGT
25401	ACAGCAGCGG TGTCGTCGCC	CCACACAGAA GGTGTGTCTT	GCAAAGGCGA CGTTTCCGCT	CCGGATAGCA GGCCTATCGT	AGACTCTGAC TCTGAGACTG
25451	AAAGCCCAAG TTTCGGGTTC	AAATCCACAG TTTAGGTGTC	CGGCGGCAGC GCCGCCGTCG	AGCAGGAGGA TCGTCCTCCT	GGAGCGCTGC CCTCGCGACG
25501	GTCTGGCGCC CAGACCGCGG	CAACGAACCC GTTGCTTGGG	GTATCGACCC CATAGCTGGG	GCGAGCTTAG CGCTCGAATC	AAACAGGATT TTTGTCCTAA

Figure 27 AA

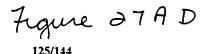
25551	ጥጥተር ርር ልር ጥር	ጥርጥልጥርርጥልጥ	ATTTCAACAG	AGCAGGGGCC	AAGAACAAGA
23331					
	AAAGGGTGAG	ACATACGATA	TAAAGTTGTC	TEGTELEGG	TTCTTGTTCT
25601	CCTCAAAATA	AAAAACAGGT	CTCTGCGATC	CCTCACCCGC	AGCTGCCTGT
25002					
	CGACTTTTAT	TTTTTGTCCA	GAGACGCTAG	GGAGTGGGCG	TUGAUGGACA
25651	ATCACAAAAG	CCAACATCAG	CTTCGCCGCA	CCCTCGAAGA	CCCCCACCCT
23031					
	TAGTGTTTTC	GCTTCTAGTC	GAAGCCGCGT	GUGACUTTUT	GCGCCTCCGA
25701	СФСФФСТА	AATACTGCGC	CCTCACTCTT	AACCACTAGT	TTCCCCCCCT
20.02					
	GAGAAGTCAT	TTATGACGCG	CGACTGAGAA	TICCIGATCA	AAGCGCGGGA
25751	TTCTCAAATT	TAAGCGCGAA	AACTACGTCA	TCTCCAGCGG	CCACACCCGG
			-		
	AAGAGTTTAA	ATTCGCGCTT	TIGATGCAGT	AGAGGTCGCC	GGTGTGGGCC
25801	CGCCAGCACC	TGTTGTCAGC	GCCATTATGA	GCAAGGAAAT	TCCCACGCCC
	GCGG1CG1GG	ACAACAGTCG	CGGTAATACT	CGIICCITIA	WGGG1GCGG
				•	
25851	TACATGTGGA	GTTACCAGCC	ACAAATGGGA	CTTGCGGCTG	GAGCTGCCCA
		CAATGGTCGG			
	AIGIACACCI	CWWIGGICGG	IGITIACCCI	GAACGCCGAC	CICOACGGI
25901	AGACTACTCA	ACCCGAATAA	ACTACATGAG	CGCGGGACCC	CACATGATAT
		TGGGCTTATT			
	ICIGNIGNGI	1000011711	IGNIGIACIC	000000	GIGIACIAIA
25951	CCCGGGTCAA	CGGAATACGC	GCCCACCGAA	ACCGAATTCT	CCTGGAACAG
	GGGCCCAGTT	GCCTTATGCG	ССССТСССТТ	TCCCTTAACA	GCACCTTCTC
	GGGCCCAG11	GCC11A1GCG	C000100C11	. 00011121011	0030011010
26001	GCGGCTATTA	CCACCACACC	TCGTAATAAC	CTTAATCCCC	GTAGTTGGCC
	CGCCGATAAT	GGTGGTGTGG	AGCATTATTG	GAATTAGGGG	CATCAACCGG
	00000				
26051		GTGTACCAGG			
	GCGACGGGAC	CACATGGTCC	TTTCAGGGCG	AGGGTGGTGA	CACCATGAAG
26101	CCAGAGACGC	CCAGGCCGAA	GTTCAGATGA	CTAACTCAGG	GGCGCAGCTT
	GGTCTCTGCG	GGTCCGGCTT	CAAGTCTACT	GATTGAGTCC	CCGCGTCGAA
				0000000000	0010110001
26151	GCGGGCGGCT	TTCGTCACAG	GGTGCGGTCG	CCCGGGCAGG	GTATAACTCA
	CGCCCGCCGA	AAGCAGTGTC	CCACGCCAGC	GGGCCCGTCC	CATATTGAGT
26201	CCDCACAADC	AGAGGGCGAG	CTATTOACCT	CAACCACCAC	TOCOTO
26201					
	GGACTGTTAG	TCTCCCGCTC	CATAAGTCGA	GTTGCTGCTC	AGCCACTCGA
26261	CCTCGCTTGG	memoremene.	CACCCCACAT	TTCACATCCC	ceececeec
20231					
	GGAGCGAACC	AGAGGCAGGC	CTGCCCTGTA	AAGTCTAGCC	GCCGCGGCCG
26301	CGCTCTTCAT	תרארפררתר פ	ተር አርርር እ አጥር	ርጥልልርጥርጥርርር	AGACCTCGTC
20301					
	GCGAGAAGTA	AGTGCGGAGC	AGITCGTTAG	GATTGAGACG	TUTGGAGCAG
26351	CTCTGAGCCG	CCCTCTCGAG	GCATTGGAAC	TCTGCAATTT	ATTGAGGAGT
	GAGACTCGGC	GCGAGACCTC	CGTAACCTTG	AGAÇÇTTAAA	TAACTCCTCA
26401	TTGTGCCATC	ССТСТАСТТТ	ልልሮሮርርሚተርጥ	CCCCACCTCC	СССССАСТАТ
20407					
	AACACGGTAG	CCAGATGAAA	TIGGGAAGA	GCCCTGGAGG	GCCGGTGATA
	•		•		•
26451	CCGGATCAAT	TTATTCCTAA	CTTTGACGCG	GTAAAGGACT	CGGCGGACGG
		AATAAGGATT			
	GGCCTAGTTA	WWIWWOOWIJ.	GAAACTGCGC	CWITTCCIGN	0 010

Figure 27 AB

26501	CTACGACTGA GATGCTGACT	ATGTTAAGTG TACAATTCAC	GAGAGGCAGA CTCTCCGTCT	GCAACTGCGC CGTTGACGCG	CTGAAACACC GACTTTGTGG
26551	TGGTCCACTG ACCAGGTGAC	TCGCCGCCAC AGCGGCGGTG	AAGTGCTTTG TTCACGAAAC	CCCGCGACTC GGGCGCTGAG	CGGTGAGTTT GCCACTCAAA
26601	ACGATGAAAC	AATTGCCCGA TTAACGGGCT	CCTAGTATAG	CTCCCGGGCC	GCGTGCCGCA
26651	GGCCGAATGG	GCCCAGGGAG CGGGTCCCTC	TCGAACGGGC	ATCGGACTAA	GCCCTCAAAT
26701	GGGTCGCGGG	CCTGCTAGTT GGACGATCAA	CTCGCCCTGT	CCCCTGGGAC	ACAAGAGTGA
26751	CACTAAACGT	ACTGTCCTAA TGACAGGATT	GGGACCTAAT	GTAGTTCTAG	AAACAACGGT
26801	AGAGACACGA	GAGTATAATA CTCATATTAT	TTATGTCTTT	AATTTTATAT	GACCCCGAGG
26851	ATAGCGGTAG	CTGTAAACGC GACATTTGCG	GTGGCAGAAG	TGGGCGGGTT	CGTTTGGTTC
26901	CGCTTGGAAT	CCTGGTACTT	AATTGTAGAG	AGGGAGACAC	TAAATGTTGT
26951	CAAAGTTGGG	AGACGGAGTG TCTGCCTCAC GAAAAAACAC	TCAGATGCTC	TCTTGGAGAG	GCTCGAGTCG
27001	ATGAGGTAGT	CTTTTTTGTG	GTGGGAGGAA	TGGACGGCCC	TTGCATGCTC
27051	ACGCAGTGGC	CGGCGACGTG	GTGTGGATGG	CGGACTGGCA	TTTGGTCTGA GAGGTGAGCT
27101	AAAAGGCCTG	TCTGGAGTTA	TTGAGACAAA	TGGTCTTGTC	CTCCACTCGA GTGGGGTTTA
27151	ATCTTTTGGG	AATCCCATAA	TCCGGTTTCC	GCGTCGATGA	CACCCAAAT
	ACTTGTTAAG	TTCGTTGAGA	TGCCCGATAA	GATTAAGTCC	AAAGAGATCT TTCTTATACT
	TAGCCCCAAC	CCCAATAAGA	GACAGAACAC	TAAGAGAAAT	AAGAATATGA ATTTGCATTT
	TTGCGAAGAG	ACGGATTCCG	AGCGGCGGAC	GACACACGTG	TAAACGTAAA
	TAACAGTCGA	AAAATTTGCG	ACCCCAGCGG	TGGGTTCTAC	TAATCCATGT
27401	ATTAGGATCO	AAATGAGTGG	GAACGCAGTC	GGGTGCCATC	GTGGGTTTTC

Figure 27AC

WO 02/022080 PCT/US01/28861 27451 GTGGATTTTA AGGAGCCAGC CTGTAATGTT ACATTCGCAG CTGAAGCTAA CACCTAAAAT TCCTCGGTCG GACATTACAA TGTAAGCGTC GACTTCGATT 27501 TGAGTGCACC ACTCTTATAA AATGCACCAC AGAACATGAA AAGCTGCTTA · ACTCACGTGG TGAGAATATT TTACGTGGTG TCTTGTACTT TTCGACGAAT 27551 TTCGCCACAA AAACAAAATT GGCAAGTATG CTGTTTATGC TATTTGGCAG AAGCGGTGTT TTTGTTTTAA CCGTTCATAC GACAAATACG ATAAACCGTC 27601 CCAGGTGACA CTACAGAGTA TAATGTTACA GTTTTCCAGG GTAAAAGTCA GGTCCACTGT GATGTCTCAT ATTACAATGT CAAAAGGTCC CATTTTCAGT 27651 TAAAACTTTT ATGTATACTT TTCCATTTTA TGAAATGTGC GACATTACCA ATTTTGAAAA TACATATGAA AAGGTAAAAT ACTTTACACG CTGTAATGGT 27701 TGTACATGAG CAAACAGTAT AAGTTGTGGC CCCCACAAAA TTGTGTGGAA ACATGTACTC GTTTGTCATA TTCAACACCG GGGGTGTTTT AACACACCTT 27751 AACACTGGCA CTTTCTGCTG CACTGCTATG CTAATTACAG TGCTCGCTTT TTGTGACCGT GAAAGACGAC GTGACGATAC GATTAATGTC ACGAGCGAAA 27801 GGTCTGTACC CTACTCTATA TTAAATACAA AAGCAGACGC AGCTTTATTG CCAGACATGG GATGAGATAT AATTTATGTT TTCGTCTGCG TCGAAATAAC 27851 AGGAAAAGAA AATGCCTTAA TTTACTAAGT TACAAAGCTA ATGTCACCAC TCCTTTTCTT TTACGGAATT AAATGATTCA ATGTTTCGAT TACAGTGGTG 27901 TAACTGCTTT ACTCGCTGCT TGCAAAACAA ATTCAAAAAG TTAGCATTAT ATTGACGAAA TGAGCGACGA ACGTTTTGTT TAAGTTTTTC AATCGTAATA 27951 AATTAGAATA GGATTTAAAC CCCCCGGTCA TTTCCTGCTC AATACCATTC TTAATCTTAT CCTAAATTTG GGGGGCCAGT AAAGGACGAG TTATGGTAAG 28001 CCCTGAACAA TTGACTCTAT GTGGGATATG CTCCAGCGCT ACAACCTTGA GGGACTTGTT AACTGAGATA CACCCTATAC GAGGTCGCGA TGTTGGAACT 28051 AGTCAGGCTT CCTGGATGTC AGCATCTGAC TTTGGCCAGC ACCTGTCCCG TCAGTCCGAA GGACCTACAG TCGTAGACTG AAACCGGTCG TGGACAGGGC 28101 CGGATTTGTT CCAGTCCAAC TACAGCGACC CACCCTAACA GAGATGACCA GCCTAAACAA GGTCAGGTTG ATGTCGCTGG GTGGGATTGT CTCTACTGGT 28151 ACACAACCAA CGCGGCCGCC GCTACCGGAC TTACATCTAC CACAAATACA TGTGTTGGTT GCGCCGGCGG CGATGGCCTG AATGTAGATG GTGTTTATGT 28201 CCCCAAGTTT CTGCCTTTGT CAATAACTGG GATAACTTGG GCATGTGGTG GGGGTTCAAA GACGGAAACA GTTATTGACC CTATTGAACC CGTACACCAC 28251 GTTCTCCATA GCGCTTATGT TTGTATGCCT TATTATTATG TGGCTCATCT CAAGAGGTAT CGCGAATACA AACATACGGA ATAATAATAC ACCGAGTAGA 28301 GCTGCCTAAA GCGCAAACGC GCCCGACCAC CCATCTATAG TCCCATCATT CGACGGATTT CGCGTTTGCG CGGGCTGGTG GGTAGATATC AGGGTAGTAA



28351 GTGCTACACC CAAACAATGA TGGAATCCAT AGATTGGACG GACTGAAACA

CACGATGTGG GTTTGTTACT ACCTTAGGTA TCTAACCTGC CTGACTTTGT

28451	TTTTATATTA AAAATATAAT	CTGACCCTTG GACTGGGAAC	TTGCGCTTTT AACGCGAAAA	TTGTGCGTGC AACACGCACG	TCCACATTGG AGGTGTAACC
28501	CTGCGGTTTC GACGCCAAAG	TCACATCGAA AGTGTAGCTT	GTAGACTGCA CATCTGACGT	TTCCAGCCTT AAGGTCGGAA	CACAGTCTAT GTGTCAGATA
28551	TTGCTTTACG AACGAAATGC	GATTTGTCAC CTAAACAGTG	CCTCACGCTC GGAGTGCGAG	ATCTGCAGCC TAGACGTCGG	TCATCACTGT AGTAGTGACA
28601	GGTCATCGCC	TTTATCCAGT	GCATTGACTG	GGTCTGTGTG CCAGACACAC	CGCTTTGCAT
28651	ATCTCAGACA	CCATCCCCAG	TACAGGGACA	GGACTATAGC CCTGATATCG	TGAGCTTCTT
28701	AGAATTCTTT	AATTATGAAA	TTTACTGTGA	CTTTTCTGCT GAAAAGACGA	GATTATTTGC
28751	ACCCTATCTG	CGTTTTGTTC	CCCGACCTCC	AAGCCTCAAA TTCGGAGTTT	GACATATATC
28801	ATGCAGATTC		GGAATATTCC	AAGTTGCTAC	
28851	GCGATCTTTC	CGAAGCCTGG	TTATATGCAA	TCATCTCTGT	TATGGTGTTC
28901	TGCAGTACCA	TCTTAGCCCT	AGCTATATAT	AGTAGAGACA CCCTACCTTG	ACATTGGCTG
28951	GAACGCAATA	AGAATCGGGA GATGCCATGA	ACCACCCAAC	TTTCCCCGCG	TGTAACCGAC
29001	TTCCACTGCA	ACAAGTTGTT	GCCGGCGGCT	AAAGGGGCGC TTGTCCCAGC	CAATCAGCCT
29051	CGCCCACCTT	CTCCCACCCC	CACTGAAATC	AACAGGGTCG AGCTACTTTA	ATCTAACAGG
29101	GCGGGTGGAA	GAGGGTGGGG TGACACCCTA	GTGACTTAG GATCTAGAAA	TCGATGAAAT TGGACGGAAT	TATTACAGAG
	TCCTCTACTG	ACTGTGGGAT	CTAGATCTTT	ACCTGCCTTA	ATAATGTCTC
	GTCGCGGACG TCAAGAGCTC	ATCTTTCTGC	GTCCCGTCGC	CGGCTCGTTG	TCGCGTACTT
	AGTTCTCGAG	GTTCTGTACC	AATTGAACGT	GGTCACGTTT	TCCCCATAGA
		TTTCGTCCGG	TTTCAGTGGA	TGCTGTCATT	ATGGTGGCCT
29301	CACCGCCTTA GTGGCGGAAT	GCTACAAGTT CGATGTTCAA	GCCAACCAAG CGGTTGGTTC	GCAGTCTTTA	ACCACCAGTA

Figure 27 A E

29401					CACCCTTATT GTGGGAATAA
29451	AAGACCCTGT TTCTGGGACA			CCCTTTAACT GGGAAATTGA	
29501				TTAGCAAATT AATCGTTTAA	
29551				CAGCTCTGGT GTCGAGACCA	
29601				AAATGGAATG TTTACCTTAC	
29651	- ·			TCATGTTGTT AGTACAACAA	
29701				CCCGTGTATC GGGCACATAG	
29751				TACTCCTCCC ATGAGGAGGG	
29801				TACTCTCTTT ATGAGAGAAA	
29851				GCGCTCAAAA CGCGAGTTTT	
29901				CTCCCAAAAT GAGGGTTTTA	
29951				ACATAAACCT TGTATTTGGA	
30001	GCACCCTCA CGTGGGGAGT			ACTGTGGCTG TGACACCGAC	
30051				GCAATCACAG CGTTAGTGTC	
30101	CCGTGCACGA GGCACGTGCT				
30151	TCAGAAGGAA AGTCTTCCTT	-	-		
30201	TAGCAGTACC ATCGTCATGG				
30251	GTAGCTTGGG CATCGAACCC				

Figure 27 AF

30351	TTTGACCGTA	GCAACTGGTC	CAGGTGTGAC	TATTAATAAT	ACTTCCTTGC
		CGTTGACCAG			
30401	AAACTAAAGT	TACTGGAGCC	TTGGGTTTTG	ATTCACAAGG	CAATATGCAA
	TTTGATTTCA	ATGACCTCGG	AACCCAAAAC	TAAGTGTTCC	GTTATACGTT
30451	CTTAATGTAG	CAGGAGGACT	AAGGATTGAT	TCTCAAAACA	GACGCCTTAT
		GTCCTCCTGA			
30501	ACTTGATGTT	AGTTATCCGT	TTGATGCTCA	AAACCAACTA	AATCTAAGAC
		TCAATAGGCA			
30551	TAGGACAGGG	CCCTCTTTTT	ATAAACTCAG	CCCACAACTT	GGATATTAAC
		GGGAGAAAA			
30601	TACAACAAAG	GCCTTTACTT	GTTTACAGCT	TCAAACAATT	CCAAAAAGCT
		CGGAAATGAA			·
30651	TGAGGTTAAC	CTAAGCACTG	CCAAGGGGTT	GATGTTTGAC	GCTACAGCCA
		GATTCGTGAC			
30701	TAGCCATTAA	TGCAGGAGAT	GGGCTTGAAT	TTGGTTCACC	TAATGCACCA
		ACGTCCTCTA			
30751	AACACAAATC	CCCTCAAAAC	AAAAATTGGC	CATGGCCTAG	AATTTGATTC
		GGGAGTTTTG			
30801	AAACAAGGCT	ATGGTTCCTA	AACTAGGAAC	TGGCCTTAGT	TTTGACAGCA
		TACCAAGGAT	•		
30851	CAGGTGCCAT	TACAGTAGGA	AACAAAAATA	ATGATAAGCT	AACTTTGTGG
		ATGTCATCCT			
30901	ACCACACCAG	CTCCATCTCC	TAACTGTAGA	CTAAATGCAG	AGAAAGATGC
		GAGGTAGAGG			
30951	TAAACTCACT	TTGGTCTTAA	CAAAATGTGG	CAGTCAAATA	CTTGCTACAG
		AACCAGAATT			
31001	TTTCAGTTTT	GGCTGTTAAA	GGCAGTTTGG	CTCCAATATC	TGGAACAGTT
		CCGACAATTT			
31051	CAAAGTGCTC	ATCTTATTAT	AAGATTTGAC	GAAAATGGAG	TGCTACTAAA
	GTTTCACGAG				
31101	CAATTCCTTC	CTGGACCCAG	AATATTGGAA	CTTTAGAAAT	GGAGATCTTA
					CCTCTAGAAT
31151	CTGAAGGCAC	AGCCTATACA	AACGCTGTTG	GATTTATGCC	TAACCTATCA
	GACTTCCGTG	TCGGATATGT	TTGCGACAAC	CTAAATACGG	ATTGGATAGT
31201	GCTTATCCAA	AATCTCACGG	TAAAACTGCC	AAAAGTAACA	TTGTCAGTCA
	CGAATAGGTT	TTAGAGTGCC	ATTTTGACGG	TTTTCATTGT	AACAGTCAGT

Figure 27 AB

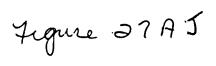
				1 € 1/65
31251	=			ACCATTACAC TGGTAATGTG
31301				ATACTCTATG
				TATGAGATAC
31351	TCATTTTCAT AGTAAAAGTA			AAATATTTGC TTTATAAACG
31401	CACATCCTCT GTGTAGGAGA	 	CCAAGAATAA GGTTCTTATT	
31451			TTGCAGAAAA	
31501	TTTTTCATTC		AACGTCTTTT	
31301		 	TGTATCGAAT	
31551		 	TATTCAACCT ATAAGTTGGA	
31601		 	CCCCGGCTGG	
31651			AGGTGTTATA	
		 	TCCACAATAT	
31701			TATTAATAAA ATAATTATTT	
31751			TGCTGAGCCA ACGACTCGGT	
31801			AGGAGAAGTC TCCTCTTCAG	
31851		 	TAGGGCGGTG ATCCCGCCAC	
31901			TCCGTCCTGC AGGCAGGACG	
31951	CATGGCAGTG GTACCGTCAC	 		
32001	GCCTTGTCCT CGGAACAGGA		TGATCTCACT ACTAGAGTGA	
32051	CAGTAACTGC GTCATTGACG			
32101	GGCGCTGTAT CCGCGACATA	 		
	CATACCACAA GTATGGTGTT			

Figure 27 AH

32251				ATCCACCACC TAGGTGGTGG	
32301				ACTGCAGGGA TGACGTCCCT	
32351				TAACCATGGA ATTGGTACCT	
32401				GCACACGTGC CGTGTGCACG	
32451				CCATATCCCA GGTATAGGGT	
32501				CAGGGAAGAC GTCCCTTCTG	
32551				TTCGGGCAGC AAGCCCGTCG	
32601				CAAAAGGAGG GTTTTCCTCC	
32651				GATCGTGTTG CTAGCACAAC	
32701				ATTTCCTGAA TAAAGGACTT	
32751				CGGTCTCGCC GCCAGAGCGG	
32801				TCTCAAAGCA AGAGTTTCGT	
32851				CATGCGCCGC GTACGCGGCG	
32901	ACATCCACCA TGTAGGTGGT	CCGCAGAATA GGCGTCTTAT	AGCCACACCC TCGGTGTGGG	AGCCAACCTA TCGGTTGGAT	CACATTCGTT GTGTAAGCAA
32951	CTGCGAGTCA GACGCTCAGT	CACACGGGAG GTGTGCCCTC	GAGCGGGAAG CTCGCCCTTC	AGCTGGAAGA TCGACCTTCT	ACCATGTTTT TGGTACAAAA
33001				TCAAAATGAA AGTTTTACTT	
33051	GTGAACGCGC CACTTGCGCG	TCCCCTCCGG AGGGGAGGCC	TGGCGTGGTC ACCGCACCAG	AAACTCTACA TTTGAGATGT	GCCAAAGAAC CGGTTTCTTG
33101	AGATAATGGC TCTATTACCG	ATTTGTAAGA TAAACATTCT	TGTTGCACAA ACAACGTGTT	TGGCTTCCAA ACCGAAGGTT	AAGGCAAACG TTCCGTTTGC

Figure 27 AI

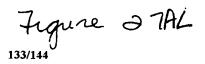
33201	CTCTATAAAC GAGATATTTG			CGGGTTTATT	
33251				CCCGAATATT GGGCTTATAA	•
33301				: ACCTTCAGCC : TGGAAGTCGG	
33351	AATCATGATT TTAGTACTAA			CAGACCTGTA GTCTGGACAT	
33401				CCGTAGGTCC GGCATCCAGG	
33451	CCAGCTGAAC	ATAATCGTGC	AGGTCTGCAC	GGACCAGCGC CCTGGTCGCG	GGCCACTTCC
33501				CTGATTATGA GACTAATACT	
33551	•			GTAAGCTTGT CATTCGAACA	
33601				AATCAGGCAA TTAGTCCGTT	
33651				TGCAGATAAA ACGTCTATTT	
33701				TTTTCTCTCA AAAAGAGAGT	
33751		•		ACAAAAAAAC TGTTTTTTTG	
33801				CCCTTATAAG GGGAATATTC	
33851				AACTGGTCAC TTGACCAGTG	
33901	AAGCACCACC	GACAGCTCCT	CGGTCATGTC		ATGTAAGACT
33951	CGGTAAACAC GCCATTTGTG	ATCAGGTTGA	TTCACATCGG	TCAGTGCTAA	AAAGCGACCG
34001	AAATAGCCCG	GGGGAATACA	TACCCGCAGG	CGTAGAGACA	ACATTACAGC
34051	CCCCATAGGA	GGTATAACAA	aattaatagg	AGAGAAAAAC	ACATAAACAC
	20001711001				



34151	ACATACAGCG TGTATGTCGC	CTTCCACAGC GAAGGTGTCG	GGCAGCCATA CCGTCGGTAT	ACAGTCAGCC TGTCAGTCGG	TTACCAGTAA AATGGTCATT
34201		CTATTAAAA GATAATTTTT			
34251		TAAAAAAGGG ATTTTTTCCC			
34301		TAACGGTTAA ATTGCCAATT			
34351		GCCCAGAAAC CGGGTCTTTG			
34401		CGTTTTCCCA GCAAAAGGGT			
34451		ACACATACAA TGTGTATGTT			
34501		ACGCCCCGCG TGCGGGGCGC			
			•		PacI
34551		CAATCCAAAA GTTAGGTTTT			
34601		TGCGACGCGA ACGCTGCGCT			
34651	CTCGCTTCCG GAGCGAAGGC	GCGGCATCGG CGCCGTAGCC	GATGCCCGCG CTACGGGCGC	TTGCAGGCCA AACGTCCGGT	TGCTGTCCAG ACGACAGGTC
34701		GACGACCATC CTGCTGGTAG			
34751		AAAGGCCGCG TTTCCGGCGC			
34801	CCTGACGAGC GGACTGCTCG	ATCACAAAAA TAGTGTTTTT	TCGACGCTCA AGCTGCGAGT	AGTCAGAGGT TCAGTCTCCA	GGCGAAACCC CCGCTTTGGG
34851	GACAGGACTA CTGTCCTGAT	TAAAGATACC ATTTCTATGG	AGGCGTTTCC TCCGCAAAGG	CCCTGGAAGC GGGACCTTCG	TCCCTCGTGC AGGGAGCACG
34901	GCTCTCCTGT CGAGAGGACA	TCCGACCCTG AGGCTGGGAC	CCGCTTACCG GGCGAATGGC	GATACCTGTC CTATGGACAG	CGCCTTTCTC GCGGAAAGAG
34951			mm0m01m100	ጥርልርርርጥርጥል	CCTATCTCAC
	CCTTCGGGAA GGAAGCCCTT	GCGTGGCGCT			

Figure 27 AK

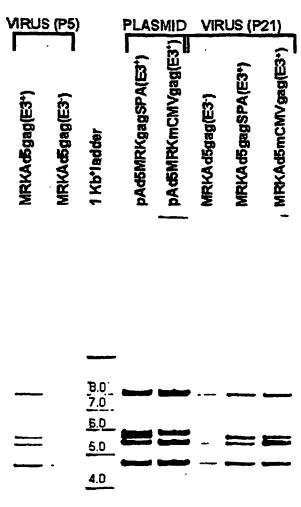
	AAGTCGGGCT	GCCGACGCGG	AATAGGCCAT	TGATAGCAGA	ACTCAGGTTG
35101	CCGGTAAGAC	ACGACTTATO	GCCACTGGCA	GCAGCCACTG	GTAACAGGAT
••••					CATTGTCCTA
35151	TAGCAGAGCG	AGGTATGTAG	GCGGTGCTAC	AGAGTTCTTG	AAGTGGTGGC
					TTCACCACCG
35201	CTAACTACGG	CTACACTAGA	AGGACAGTAT	TTGGTATCTG	CGCTCTGCTG
					GCGAGACGAC
35251	AAGCCAGTTA	CCTTCGGAAA	AAGAGTTGGT	AGCTCTTGAT	CCGGCAAACA
	TTCGGTCAAT	GGAAGCCTTT	TTCTCAACCA	TCGAGAACTA	GGCCGTTTGT
35301	AACCACCGCT	GGTAGCGGTG	GTTTTTTTGT	TTGCAAGCAG	CAGATTACGC
	TTGGTGGCGA	CCATCGCCAC	CAAAAAAAACA	AACGTTCGTC	GTCTAATGCG
35351	GCAGAAAAA	AGGATCTCAA	GAAGATCCTT	TGATCTTTTC	TACGGGGTCT
	CGTCTTTTTT	TCCTAGAGTT	CTTCTAGGAA	ACTAGAAAAG	ATGCCCCAGA
35401	GACGCTCAGT	GGAACGAAAA	CTCACGTTAA	GGGATTTTGG	TCATGAGATT
	CTGCGAGTCA	CCTTGCTTTT	GAGTGCAATT	CCCTAAAACC	AGTACTCTAA
35451	ATCAAAAAGG	ATCTTCACCT	AGATCCTTTT	AAATCAATCT	AAAGTATATA
	TAGTTTTTCC	TAGAAGTGGA	TCTAGGAAAA	TTTAGTTAGA	TTTCATATAT
35501	TGAGTAAACT	TGGTCTGACA	GTTACCAATG	CTTAATCAGT	GAGGCACCTA
	ACTCATTTGA	ACCAGACTGT	CAATGGTTAC	GAATTAGTCA	CTCCGTGGAT
35551	TCTCAGCGAT	CTGTCTATTT	CGTTCATCCA	TAGTTGCCTG	ACTCCCCGTC
	AGAGTCGCTA	GACAGATAAA	GCAAGTAGGT	ATCAACGGAC	TGAGGGGCAG
35601	GTGTAGATAA	CTACGATACG	GGAGGGCTTA	CCATCTGGCC	CCAGTGCTGC
	CACATCTATT	GATGCTATGC	CCTCCCGAAT	GGTAGACCGG	GGTCACGACG
35651	AATGATACCG	CGAGACCCAC	GCTCACCGGC	TCCAGATTTA	TCAGCAATAA
	TTACTATGGC	GCTCTGGGTG	CGAGTGGCCG	AGGTCTAAAT	AGTCGTTATT
35701		CGGAAGGGCC			
	TGGTCGGTCG	GCCTTCCCGG	CTCGCGTCTT	CACCAGGACG	TTGAAATAGG
35751	GCCTCCATCC				
	CGGAGGTAGG	TCAGATAATT	AACAACGGCC	CTTCGATCTC	ATTCATCAAG
35801	GCCAGTTAAT				
	CGGTCAATTA	TCAAACGCGT	TGCAACAACG	GTAACGATGT	CCGTAGCACC
35851	TGTCACGCTC				
	ACAGTGCGAG	CAGCAAACCA	TACCGAAGTA	AGTCGAGGCC	AAGGGTTGCT
	TCAAGGCGAG				
	AGTTCCGCTC	AATGTACTAG	GGGGTACAAC	ACGTTTTTTC	GCCAATCGAG
	CTTCGGTCCT		-		
	GAAGCCAGGA	GGCTAGCAAC	AGTCTTCATT	CAACCGGCGT	CACAATAGTG



36051	AGATGCTTTT	CTGTGACTGG	TGAGTACTCA	ACCAAGTCAT	TCTGAGAATA
	TCTACGAAAA	GACACTGACC	ACTCATGAGT	TGGTTCAGTA	AGACTCTTAT
36101	GTGTATGCGG	CGACCGAGTT	GCTCTTGCCC	GGCGTCAACA	CGGGATAATA
	CACATACGCC	GCTGGCTCAA	CGAGAACGGG	CCGCAGTTGT	GCCCTATTAT
36151	CCGCGCCACA	TAGCAGAACT	TTAAAAGTGC	TCATCATTGG	AAAACGTTCT
				AGTAGTAACC	
36201	TCGGGGCGAA	AACTCTCAAG	GATCTTACCG	CTGTTGAGAT	CCAGTTCGAT
				GACAACTCTA	
36251	GTAACCCACT	CGTGCACCCA	ACTGATCTTC	AGCATCTTTT	ACTTTCACCA
				TCGTAGAAAA	
36301	GCGTTTCTGG	GTGAGCAAAA	ACAGGAAGGC	AAAATGCCGC	AAAAAAGGGA
•)	TTTTTTCCCT
36351	ATAAGGCCGA	CACGGAAATG	TTGAATACTC	ATACTCTTCC	TTTTTCAATA
				TATGAGAAGG	
36401				CATGAGCGGA	
				GTACTCGCCT	
36451	AATGTATTTA	GAAAAATAAA	CAAATAGGGG	TTCCGCGCAC	ATTTCCCCGA
				AAGGCGCGTG	
36501	AAAGTGCCAC	CTGACGTCTA	AGAAACCATT	ATTATCATGA	CATTAACCTA
				TAATAGTACT	
36551	TAAAAATAGG	CGTATCACGA	GGCCCTTTCG	TCTTCAAGAA	TTGGATCCGA
·	ATTTTTATCC	GCATAGTGCT	CCGGGAAAGC	AGAAGTTCTT	AACCTAGGCT
		PacI			
36601	አ ጥጥር ጥጥ አ አ ጥጥ	тсттааттаа	(SEO ID NO	: 34)	

36601 ATTCTTAATT TCTTAATTAA (SEQ ID NO:34)
TAAGAATTAA AGAATTAATT (SEQ ID NO:35)

Tigure 27 AM



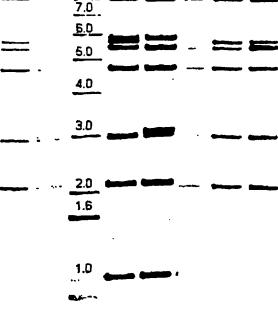


FIGURE 28

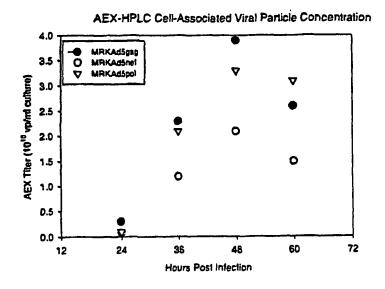


FIGURE 29A

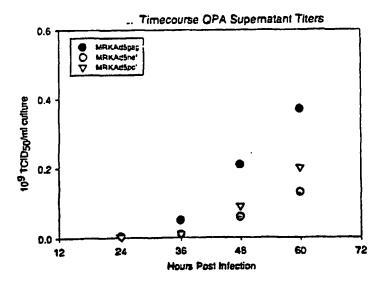


FIGURE 29B

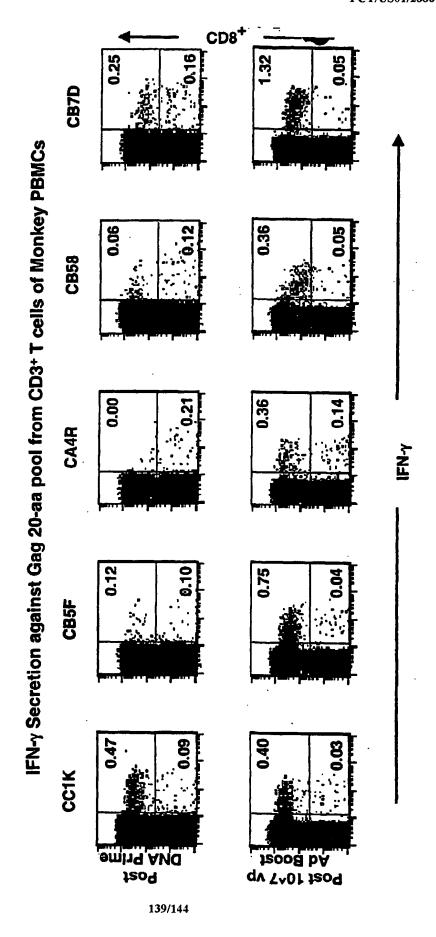
			atg Met													48	
			gtt Val 20													96	
			agg Arg													144	
			cag Gln													192	
			ctg Leu													240	
			aag Lys													288	
			gag Glu 100													336	
			Gly ggc													384	
cag Gln	aac Asn 130	ctc Leu	cag Gln	Gly ggc	cag Gln	atg Met 135	gtg Val	cac His	cag Gln	gcc Ala	atc Ile 140	tcc Ser	ccc Pro	cgg Arg	acc	432	
			tgg Trp													480	
			atg Met													528	
			atg Met 180													576	
atg Met	ctg Leu	aag Lys 195	gag Glu	acc Thr	atc Ile	aat Asn	gag Glu 200	Glu	gct Ala	gct Ala	gag Glu	tgg Trp 205	gac A sp	Arg	ctg Leu	624	
Cat His	cct Pro 210	gtg Val	cac His	gct Ala	Gly	Pro 215	att Ile	gcc	CCC Pro	Gly	cag Gln 220	atg Met	agg Arg	gag Glu	ccc Pro	672	
agg Arg 225	G]Å ggc	tct Ser	gac A sp	att Ile	gct Ala 230	ggc Gly	acc Thr	acc Thr	tcc Ser	acc Thr 235	ctc Leu	cag Gln	gag Glu	cag Gln	att Ile 240	720	
ggc Gly	tgg Trp	atg Met	acc Thr	aac Asn 245	aac Asn	ecc Pro	ccc Pro	atc Ile	cct Pro 250	gtg Val	Gly	gaa Glu	atc Ile	tac Tyr 255	aag Lys	768	

Figure 30'A"

agg Arg	tgg Trp	atc Ile	atc Ile 260	ctg Leu	ggc Gly	ctg Leu	aac Asn	aag Lys 265	att Ile	gtg Val	agg Arg	atg Met	tac Tyr 270	tcc Ser	CCC Pro	816
													ttc Phe			864
tat Tyr	gtg Val 290	gac Asp	agg Arg	ttc Phe	tac Tyr	aag Lys 295	acc Thr	ctg Leu	agg Arg	gct Ala	gag Glu 300	cag Gln	gcc Ala	tcc Ser	cag Gln	912
													aat Asn			960
cct Pro	gac Asp	tgc Cys	aag Lys	acc Thr 325	atc Ile	ctg Leu	aag Lys	gcc Ala	ctg Leu 330	ggc	ect Pro	gct Ala	gcc Ala	acc Thr 335	Ctg Leu	1008
gag Glu	gag Glu	atg Met	atg Met 340	aca Thr	gcc Ala	tgc Cys	cag Gln	999 Gly 345	gtg Val	CJA BBB	ggc	ect Pro	ggt Gly 350	cac Kis	aag Lys	1056
gcc Ala	agg Arg	gtg Val 355	ctg Leu	gct Ala	gag Glu	gcc Ala	atg Met 360	tcc Ser	cag Gln	gtg Val	acc Thr	aac Asn 365	tcc Ser	gcc Ala	acc Thr	1104
													aca Thr			1152
tgc Cys 385	Phe	aac Asn	tgt Cys	Gly	aag Lys 390	gtg Val	ggc Gly	cac His	att Ile	gcc Ala 395	aag Lys	aac Asn	tgt Cys	ágg Arg	gcc Ala 400	1200
													cac His			1248
aag Lys	gac Asp	tgc Cys	aat Asn 420	gag Glu	agg Arg	cag Gln	gcc Ala	aac Asn 425	ttc Phe	ctg Leu	<u>C</u> ly	aaa Lys	atc Ile 430	tgg Trp	ecc Pro	1296
tcc Ser	cac His	aag Lys 4 35	ggc Gly	agg Arg	cct Pro	Gly	aac Asn 440	ttc Phe	ctc Leu	cag Gln	tcc Ser	agg Arg 445	cct Pro	gag Glu	CCC Pro	1344
										Gly			aag Lys			1392
ecc Pro 465	agc Ser	cag Gln	aag Lys	cag Gln	gag Glu 470	ccc Pro	att Ile	gac Asp	aag Lys	gag Glu 475	ctg Leu	tac Tyr	Pro	ctg Leu	gcc Ala 480	1440
tcc Ser	ctg Leu	agg Arg	tcc Ser	ctg Leu 485	ttt Phe	ggc Gly	aac Asn	gac Asp	ccc Pro 490	tcc Ser	tcc Ser	cag Gln	taa	(SI	D NO:36) D NO:37)	1482

Figure 30 B

Figure 31



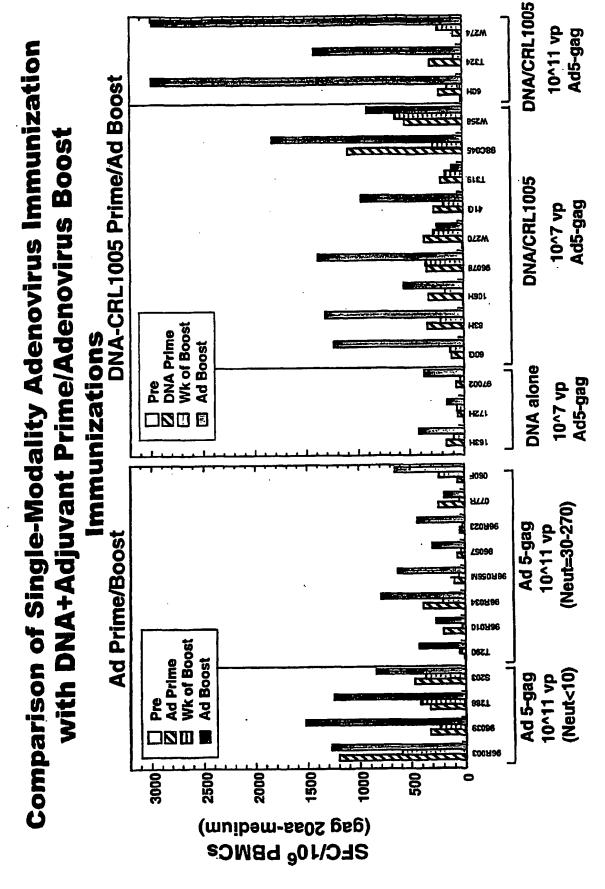


FIGURE 33A

ATGGGTGCTA	GGGCTTCTGT	GCTGTCTGGT	GGTGAGCTGG	ACAAGTGGGA	GAAGATCAGG
CTGAGGCCTG	GTGGCAAGAA	GAAGTACAAG	CTAAAGCACA	TTGTGTGGGC	CTCCAGGGAG
CTGGAGAGGT	TTGCTGTGAA	CCCTGGCCTG	CTGGAGACCT	CTGAGGGGTG	CAGGCAGATC
CTGGGCCAGC	TCCAGCCCTC	CCTGCAAACA	GGCTCTGAGG	AGCTGAGGTC	CCTGTACAAC
ACAGTGGCTA	CCCTGTACTG	TGTGCACCAG	AAGATTGATG	TGAAGGACAC	CAAGGAGGCC
CTGGAGAAGA	TTGAGGAGGA	GCAGAACAAG	TCCAAGAAGA	AGGCCCAGCA	GGCTGCTGCT
GGCACAGGCA	ACTCCAGCCA	GGTGTCCCAG	AACTACCCCA	TTGTGCAGAA	CCTCCAGGGC
CAGATGGTGC	ACCAGGCCAT	CTCCCCCGG	ACCCTGAATG	CCTGGGTGAA	GGTGGTGGAG
GAGAAGGCCT	TCTCCCCTGA	GGTGATCCCC	ATGTTCTCTG	CCCTGTCTGA	GGGTGCCACC
CCCCAGGACC	TGAACACCAT	GCTGAACACA	GTGGGGGGCC	ATCAGGCTGC	CATGCAGATG
CTGAAGGAGA	CCATCAATGA	GGAGGCTGCT	GAGTGGGACA	GGCTGCATCC	TGTGCACGCT
GGCCCCATTG	CCCCCGGCCA	GATGAGGGAG	CCCAGGGGCT	CTGACATTGC	TGGCACCACC
TCCACCCTCC	AGGAGCAGAT	TGGCTGGATG	ACCAACAACC	CCCCATCCC	TGTGGGGGAA
ATCTACAAGA	GGTGGATCAT	CCTGGGCCTG	AACAAGATTG	TGAGGATGTA	CTCCCCCACC
TCCATCCTGG	ACATCAGGCA	GGGCCCCAAG	GAGCCCTTCA	GGGACTATGT	GGACAGGTTC
TACAAGACCC	TGAGGGCTGA	GCAGGCCTCC	CAGGAGGTGA	AGAACTGGAT	GACAGAGACC
CTGCTGGTGC	AGAATGCCAA	CCCTGACTGC	AAGACCATCC	TGAAGGCCCT	GGGCCCTGCT
GCCACCCTGG	AGGAGATGAT	GACAGCCTGC	CAGGGGGTGG	GGGCCCTGG	TCACAAGGCC
AGGGTGCTGG	CTGAGGCCAT	GTCCCAGGTG	ACCAACTCCG	CCACCATCAT	GATGCAGAGG
GGCAACTTCA	GGAACCAGAG	GAAGACAGTG	AAGTGCTTCA	ACTGTGGCAA	GGTGGGCCAC
ATTGCCAAGA	ACTGTAGGGC	CCCCAGGAAG	AAGGGCTGCT	GGAAGTGTGG	CAAGGAGGC
CACCAGATGA	AGGACTGCAA	TGAGAGGCAG	GCCAACTTCC	TGGGCAAAAT	CTGGCCCTCC
CACAAGGGCA	GGCCTGGCAA	${\tt CTTCCTCCAG}$	$\mathtt{TCCAGGCCTG}$	AGCCCACAGC	CCCTCCCGAG
GAGTCCTTCA	GGTTTGGGGA	GGAGAAGACC	ACCCCCAGCC	AGAAGCAGGA	GCCCATTGAC
AAGGAGCTGT	ACCCCCTGGC	CTCCCTGAGG	TCCCTGTTTG	GCAACGACCC	CTCCTCCCAG
ATGGCTCCCA	TCTCCCCAT	TGAGACTGTG	CCTGTGAAGC	TGAAGCCTGG	CATGGATGGC
CCCAAGGTGA	AGCAGTGGCC	CCTGACTGAG	GAGAAGATCA	AGGCCCTGGT	GGAAATCTGC
ACTGAGATGG	AGAAGGAGGG	CAAAATCTCC	AAGATTGGCC	CCGAGAACCC	CTACAACACC
CCTGTGTTTG	CCATCAAGAA	GAAGGACTCC	ACCAAGTEGA	GGAAGCTGGT	GGACTTCAGG
GAGCTGAACA	AGAGGACCCA	GGACTTCTGG	GAGGTGCAGC	TGGGCATCCC	CCACCCCGCT
GGCCTGAAGA	AGAAGAAGTC	TGTGACTGTG	CTGGCTGTGG	GGGATGCCTA	CTTCTCTGTG
	AGGACTTCAG				
	TCAGGTACCA				
	CCTCCATGAC				
	AGTACATGGC				
	TTGAGGAGCT				
	AGAAGGAGCC				
	AGCCCATTGT				
					GGTGAGGCAG
					GACTGAGGAG
GCTGAGCTGG	AGCTGGCTGA	GAACAGGGAG	ATCCTGAAGG	AGCCTGTGCA	TGGGGTGTAC

FIGURE 33B

m>mc>ccccm	CCAAGGACCT	СУЛТССТСУС	ATCCAGAAGC	AGGGCCAGGG	CCAGTGGACC
	ACCAGGAGCC				
	CCAATGATGT				
	TCTGGGGCAA				
	GGACTGAGTA				
ACCCCCCCC	TGGTGAAGCT	GTGGTACCAG.	CTGGAGAAGG	AGCCCATTGT	GGGGGCTGAG
ACCTTCTATG	TGGCTGGGGC	TGCCAACAGG	GAGACCAAGC	TGGGCAAGGC	TGGCTATGTG
ACCAACAGGG	GCAGGCAGAA	GGTGGTGACC	CTGACTGACA	CCACCAACCA	GAAGACTGCC
CTCCAGGCCA	TCTACCTGGC	CCTCCAGGAC	TCTGGCCTGG	AGGTGAACAT	TGTGACTGCC
TCCCAGTATG	CCCTGGGCAT	CATCCAGGCC	CAGCCTGATC	AGTCTGAGTC	TGAGCTGGTG
AACCAGATCA	TTGAGCAGCT	GATCAAGAAG	GAGAAGGTGT	ACCTGGCCTG	GGTGCCTGCC
CACAAGGGCA	TTGGGGGCAA	TGAGCAGGTG	GACAAGCTGG	TGTCTGCTGG	CATCAGGAAG
GTGCTGTTCC	TGGATGGCAT	TGACAAGGCC	CAGGATGAGC	ATGAGAAGTA	CCACTCCAAC
TGGAGGGCTA	TGGCCTCTGA	CTTCAACCTG	CCCCTGTGG	TGGCTAAGGA	GATTGTGGCC
TCCTGTGACA	AGTGCCAGCT	GAAGGGGGAG	GCCATGCATG	GGCAGGTGGA	CTGCTCCCCT
	AGCTGGCCTG				
GTGGCCTCCG	GCTACATTGA	GGCTGAGGTG	ATCCCTGCTG	AGACAGGCCA	GGAGACTGCC
TACTTCCTGC	TGAAGCTGGC	TGGCAGGTGG	CCTGTGAAGA	CCATCCACAC	TGCCAATGGC
TCCAACTTCA	CTGGGGCCAC	AGTGAGGGCT	GCCTGCTGGT	GGGCTGGCAT	CAAGCAGGAG
TTTGGCATCC	CCTACAACCC	CCAGTCCCAG	GGGGTGGTGG	CCTCCATGAA	CAAGGAGCTG
AAGAAGATCA	TTGGGCAGGT	GAGGGACCAG	GCTGAGCACC	TGAAGACAGC	TGTGCAGATG
GCTGTGTTCA	TCCACAACTT	CAAGAGGAAG	GGGGGCATCG	GGGGCTACTC	CGCTGGGGAG
AGGATTGTGG	ACATCATTGC	CACAGACATC	CAGACCAAGG	AGCTCCAGAA	GCAGATCACC
					GAAGGCCCT
					TGACATCAAG
GTGGTGCCCA	GGAGGAAGGC	CAAGATCATC	AGGGACTATG	GCAAGCAGAT	GGCTGGGGAT
GACTGTGTGG	CCTCCAGGCA	GGATGAGGAC	TAA		
SEQ ID NO:	38				

FIGURE 34A

Met Gly Ala Arg Ala Ser Val Leu Ser Gly Gly Glu Leu Asp Lys Trp Glu Lys Ile Arg Leu Arg Pro Gly Gly Lys Lys Lys Tyr Lys Leu Lys His Ile Val Trp Ala Ser Arg Glu Leu Glu Arg Phe Ala Val Asn Pro Gly Leu Leu Glu Thr Ser Glu Gly Cys Arg Gln Ile Leu Gly Gln Leu Gln Pro Ser Leu Gln Thr Gly Ser Glu Glu Leu Arg Ser Leu Tyr Asn Thr Val Ala Thr Leu Tyr Cys Val His Gln Lys Ile Asp Val Lys Asp Thr Lys Glu Ala Leu Glu Lys Ile Glu Glu Glu Gln Asn Lys Ser Lys Lys Ala Gln Gln Ala Ala Gly Thr Gly Asn Ser Ser Gln Val Ser Gln Asn Tyr Pro Ile Val Gln Asn Leu Gln Gly Gln Met Val His Gln Ala Ile Ser Pro Arg Thr Leu Asn Ala Trp Val Lys Val Val Glu Glu Lys Ala Phe Ser Pro Glu Val Ile Pro Met Phe Ser Ala Leu Ser Glu Gly Ala Thr Pro Gln Asp Leu Asn Thr Met Leu Asn Thr Val Gly Gly His Gln Ala Ala Met Gln Met Leu Lys Glu Thr Ile Asn Glu Glu Ala Ala Glu Trp Asp Arg Leu His Pro Val His Ala Gly Pro Ile Ala Pro Gly Gln Met Arg Glu Pro Arg Gly Ser Asp Ile Ala Gly Thr Thr Ser Thr Leu Gln Glu Gln Ile Gly Trp Met Thr Asn Asn Pro Pro Ile Pro Val Gly Glu Ile Tyr Lys Arg Trp Ile Ile Leu Gly Leu Asn Lys Ile Val Arg Met Tyr Ser Pro Thr Ser Ile Leu Asp Ile Arg Gln Gly Pro Lys Glu Pro Phe Arg Asp Tyr Val Asp Arg Phe Tyr Lys Thr Leu Arg Ala Glu Gln Ala Ser Gln Glu Val Lys Asn Trp Met Thr Glu Thr Leu Leu Val Gln Asn Ala Asn Pro Asp Cys Lys Thr Ile Leu Lys Ala Leu Gly Pro Ala Ala Thr Leu Glu Glu Met Met Thr Ala Cys Gln Gly Val Gly Gly Pro Gly His Lys Ala Arg Val Leu Ala Glu Ala Met Ser Gln Val Thr Asn Ser Ala Thr Ile Met Met Gln Arg Gly Asn Phe Arg Asn Gln Arg Lys Thr Val Lys Cys Phe Asn Cys Gly Lys Val Gly His Ile Ala Lys Asn Cys Arg Ala Pro Arg Lys Lys Gly Cys Trp Lys Cys Gly Lys Glu Gly His Gln Met Lys Asp Cys Asn Glu Arg Gln Ala Asn Phe Leu Gly Lys Ile Trp Pro Ser His Lys Gly Arg Pro Gly Asn Phe Leu Gln Ser Arg Pro Glu Pro Thr Ala Pro Pro Glu Glu Ser Phe Arg Phe Gly Glu Glu Lys Thr Thr Pro Ser Gln Lys Gln Glu Pro Ile Asp Lys Glu Leu Tyr Pro Leu Ala Ser Leu Arg Ser Leu Phe Gly Asn Asp Pro Ser Ser Gln Met Ala Pro Ile Ser Pro Ile Glu Thr Val Pro Val Lys Leu Lys Pro Gly Met Asp Gly Pro Lys Val Lys Gln Trp Pro Leu Thr Glu Glu Lys Ile Lys Ala Leu Val Glu Ile Cys Thr Glu Met Glu Lys Glu Gly Lys Ile Ser Lys Ile Gly Pro Glu Asn Pro Tyr Asn Thr Pro Val Phe Ala Ile Lys Lys Lys Asp Ser Thr Lys Trp Arg Lys Leu Val Asp Phe Arg Glu Leu Asn Lys Arg Thr Gln Asp Phe Trp Glu Val Gln Leu Gly Ile Pro His Pro Ala Gly Leu Lys Lys Lys Ser Val Thr Val Leu Ala Val Gly Asp Ala Tyr Phe Ser Val Pro Leu Asp Glu Asp Phe Arg Lys Tyr Thr Ala Phe Thr Ile Pro Ser Ile Asn Asn Glu Thr Pro Gly Ile Arg Tyr Gln Tyr Asn Val Leu Pro Gln Gly Trp Lys Gly Ser Pro Ala Ile Phe Gln Ser Ser Met Thr Lys Ile Leu Glu Pro Phe Arg Lys Gln Asn Pro Asp Ile Val Ile Tyr Gln Tyr Met Ala Ala Leu Tyr Val Gly Ser Asp Leu Glu Ile Gly Gln His Arg Thr Lys Ile Glu Glu Leu Arg Gln His Leu Leu Arg Trp Gly Leu Thr Thr Pro Asp Lys Lys His Gln Lys Glu Pro Pro Phe Leu Trp Met Gly Tyr Glu Leu His Pro

FIGURE 34B

Asp Lys Trp Thr Val Gln Pro Ile Val Leu Pro Glu Lys Asp Ser Trp Thr Val Asn Asp Ile Gln Lys Leu Val Gly Lys Leu Asn Trp Ala Ser Gln Ile Tyr Pro Gly Ile Lys Val Arg Gln Leu Cys Lys Leu Leu Arg Gly Thr Lys Ala Leu Thr Glu Val Ile Pro Leu Thr Glu Glu Ala Glu Leu Glu Leu Ala Glu Asn Arg Glu Ile Leu Lys Glu Pro Val His Gly Val Tyr Tyr Asp Pro Ser Lys Asp Leu Ile Ala Glu Ile Gln Lys Gln Gly Gln Gly Gln Trp Thr Tyr Gln Ile Tyr Gln Glu Pro Phe Lys Asn Leu Lys Thr Gly Lys Tyr Ala Arg Met Arg Gly Ala His Thr Asn Asp Val Lys Gln Leu Thr Glu Ala Val Gln Lys Ile Thr Thr Glu Ser Ile Val Ile Trp Gly Lys Thr Pro Lys Phe Lys Leu Pro Ile Gln Lys Glu Thr Trp Glu Thr Trp Trp Thr Glu Tyr Trp Gln Ala Thr Trp Ile Pro Glu Trp Glu Phe Val Asn Thr Pro Pro Leu Val Lys Leu Trp Tyr Gln Leu Glu Lys Glu Pro Ile Val Gly Ala Glu Thr Phe Tyr Val Ala Gly Ala Ala Asn Arg Glu Thr Lys Leu Gly Lys Ala Gly Tyr Val Thr Asn Arg Gly Arg Gln Lys Val Val Thr Leu Thr Asp Thr Thr Asn Gln Lys Thr Ala Leu Gln Ala Ile Tyr Leu Ala Leu Gln Asp Ser Gly Leu Glu Val Asn Ile Val Thr Ala Ser Gln Tyr Ala Leu Gly Ile Ile Gln Ala Gln Pro Asp Gln Ser Glu Ser Glu Leu Val Asn Gln Ile Ile Glu Gln Leu Ile Lys Lys Glu Lys Val Tyr Leu Ala Trp Val Pro Ala His Lys Gly Ile Gly Gly Asn Glu Gln Val Asp Lys Leu Val Ser Ala Gly Ile Arg Lys Val Leu Phe Leu Asp Gly Ile Asp Lys Ala Gln Asp Glu His Glu Lys Tyr His Ser Asn Trp Arg Ala Met Ala Ser Asp Phe Asn Leu Pro Pro Val Val Ala Lys Glu Ile Val Ala Ser Cys Asp Lys Cys Gln Leu Lys Gly Glu Ala Met His Gly Gln Val Asp Cys Ser Pro Gly Ile Trp Gln Leu Ala Cys Thr His Leu Glu Gly Lys Val Ile Leu Val Ala Val His Val Ala Ser Gly Tyr Ile Glu Ala Glu Val Ile Pro Ala Glu Thr Gly Gln Glu Thr Ala Tyr Phe Leu Leu Lys Leu Ala Gly Arg Trp Pro Val Lys Thr Ile His Thr Ala Asn Gly Ser Asn Phe Thr Gly Ala Thr Val Arg Ala Ala Cys Trp Trp Ala Gly Ile Lys Gln Glu Phe Gly Ile Pro Tyr Asn Pro Gln Ser Gln Gly Val Val Ala Ser Met Asn Lys Glu Leu Lys Lys Ile Ile Gly Gln Val Arg Asp Gln Ala Glu His Leu Lys Thr Ala Val Gln Met Ala Val Phe Ile His Asn Phe Lys Arg Lys Gly Gly Jle Gly Gly Tyr Ser Ala Gly Glu Arg Ile Val Asp Ile Ile Ala Thr Asp Ile Gln Thr Lys Glu Leu Gln Lys Gln Ile Thr Lys Ile Gln Asn Phe Arg Val Tyr Tyr Arg Asp Ser Arg Asn Pro Leu Trp Lys Gly Pro Ala Lys Leu Leu Trp Lys Gly Glu Gly Ala Val Val Ile Gln Asp Asn Ser Asp Ile Lys Val Val Pro Arg Arg Lys Ala Lys Ile Ile Arg Asp Tyr Gly Lys Gln Met Ala Gly Asp Asp Cys Val Ala Ser Arg Gln Asp Glu Asp SEQ ID NO: 39

International application No.

PCT/US01/28861

IPC(7) US CL According t	SSIFICATION OF SUBJECT MATTER : C12N 15/86 : 435/456 o International Patent Classification (IPC) or to both LDS SEARCHED	national classification and IPC	
	ocumentation searched (classification system followed 424/205.1, 207.1, 227.1, 233.1; 435/69.1, 69.3, 173		
Documentat	ion searched other than minimum documentation to the	ne extent that such documents are include	d in the fields searched
	ata base consulted during the international search (national search (natio	me of data base and, where practicable, s	earch terms used)
C. DOC	UMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where a	ppropriate, of the relevant passages	Relevant to claim No.
X	WO 96/39178 (ERTL et al.) 12 December 1996 (1) and claims 1 and 5.	2.12.1996), see page 5, 6,10, 12, 13	1-3, 8-11, 18
Y			4, 5, 13-17, 29-32, 34, 35, 37
X 	US 6,019,978 A (ERTL et al.) 1 February 2000,(0	1/02/2000), see columns 2, 7 and 8.	1-3, 8-11, 18
Y			4, 5, 13-17, 29-32, 34, 35, 37
X,P	US 6,287,571 f (ERTL et al.) 11 September 200 and claim 1.	01 (11/09/2001), see columns 2, 7, 8	1, 9, 18
x	US 5,643,579A (HUNG et al.) 1 July 1997 (01/07/	(1997), see examples 1, 2, 25 and 26.	1-3, 8, 9-11, 18
Y			4,5,13-17, 29-32, 34, 35, 37
Y	WANG et al. The use of an E1-deleted, replication expressing the rabies virus glycoprotein for early v Journal of Virology (March 1997) Vol. 71, No. 5,	accination of mice against rables virus.	1-3, 9-11, 13-18
N Burther	documents are listed in the continuation of Box C.	See patent family annex.	
	pocial categories of cited documents:	"T" Ister document published after the in	Accordance (Disc. Associate
"A" documen	t defining the general state of the art which is not considered to	priority date and not in conflict with understand the principle or theory us	the application but cited to
. •	oplication or patent published on or after the international filing	"X" document of particular relevance; the considered novel or cannot be considered novel or cannot be considered novel or cannot be considered.	fered to involve an inventive
	t which may throw doubts on priority claim(s) or which is cited ish the publication date of another citation or other special reason fled)	"Y" document of particular relevance; the considered to involve an inventive se combined with one or more other au combination being obvious to a pers	tep when the document is th documents, such
	referring to an oral disclosure, use, exhibition or other means	*&* document member of the same patent	
priority	t published prior to the international filing date but later than the		
	octual completion of the international search 2002 (06.02.2002)	Date of mailing of the international sea	rch report
	ailing address of the ISA/US	Authorized officer	1,00
Con	mnissioner of Patents and Trademarks PCT	Ulrike Winkler, Ph.D.	weekins for
Wat	bington, D.C. 20231 D. (703)305-3230	Telephone No. 703-308-0196	/)
			

Form PCT/ISA/210 (second sheet) (July 1998)

International application No.

PCT/US01/28861

INTERNATIONAL SEARCH REPORT

Category •	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	NATUK et al. Immunogenicity of recombinant human adenovirus -human immunodeficiency virus vaccines in chimpanzees. Aids Research and Human Retroviruses (1993) Vol. 9, No. 5, pp395-404, see material and methods.	1, 9, 29-32
Y	PREVEC et al. Immune response to HIV-1 gag antigens induced by recombinant adenovirus vectors in mice and rhesus macaque monkeys. Journal of Acquired Immune Deficincy Syndrome. (1991) Vol. 4, No. 6 pp. 568-76, see abstract.	1, 9, 29-32
Y	LORI et al. Rapid protection against human immunodeficiency virus type 1 (HIV-1) replication mediated by high efficiency non-retroviral delivery of genes interfering with HIV-1 tat and gag. Gene Therapy (1994) Vol. 1, No. 1, pp. 27-31, see abstract.	1, 9
Y .,	PFARR et al. Differential effects of polyadenylation regions on gene expression in mammalian cells. DNA (1986) Vol. 5, No. 2, pp.115-22, see abstract.	16
Y	NATUK et al. Adenovirus vectored vaccine. Developmental Biological Standards (1994) Vol. 82, pp. 71-77, see abstract.	1, 9
	,	
į		

International application No.

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)
This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:
Claim Nos.: because they relate to subject matter not required to be searched by this Authority, namely:
Claim Nos.: because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
Claim Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).
Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)
This International Searching Authority found multiple inventions in this international application, as follows: Please See Continuation Sheet
 As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-5, 8-11, 13-18, 29-32, 34, 35, 37
Remark on Protest The additional search fees were accompanied by the applicant's protest. No protest accompanied the payment of additional search fees.

International application No.

PCT/US01/28861

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING
This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group	Claims	
1	1-5, 8-11, 13-18, 29, 30, 31, 32, 34, 35, 37	The claims are directed to an adenoviral vector that is at least partially deleted of $\Delta E1$, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Gag protein (SEQ ID NO: 29) inserted in the parallel orientation of E1. In addition the vector contains a promoter and a polyadenylation signal.
2	6, 7, 36	The claims are directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ and $\Delta E3$, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Gag protein (SEQ ID NO: 29).
3	12, 33	The claims are directed to an adenoviral vector that is at least partially deleted of ΔΕ1, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV protein inserted in the antiparallel orientation of Ε1.
4	19-23, 38-42	The claims are directed to a method of making and harvesting of a recombinant adenoviral particle that contains a gene encoding an HIV Gag protein.
5	24, 27, 28, 43, 46, 47	The claim is directed to a method of generating a cellular mediated immune response to HIV Gag protein with the recombinant adenoviral particle.
6	25, 26, 44, 45	The claim is directed to a method of generating a cellular mediated immune response to HIV Gag protein with the recombinant adenoviral particle in <u>addition to</u> administering a DNA plasmid vaccine.
7	48-51, 53, 54, 56	The claims are directed to an adenoviral vector that is at least partially deleted of <u>AE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Pol protein (SEQ ID NO: 1)</u> inserted in the parallel orientation of E1.
8	48-51, 53, 54, 56	The claims are directed to an adenoviral vector that is at least partially deleted of <u>AEI</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 5) inserted in the parallel orientation of E1.
9	48-51, 53, 54, 56	The claims are directed to an adenoviral vector that is at least partially deleted of <u>AEI</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 7) inserted in the parallel orientation of E1.
10	52	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 1) inserted in the antiparallel orientation of E1.
11	52	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 5) inserted in the antiparallel orientation of E1.
12	52	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 7) inserted in the antiparallel orientation of E1.
13	55	The claim is directed to an adenoviral vector that is at least partially deleted of ΔΕ1

International application No.

		and ΔE3, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 1)
14	55	inserted in E1. The claim is directed to an adenoviral vector that is at least partially deleted of Δ E1 and Δ E3, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ 1D NO: 5) inserted in E1.
15	55	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ and $\Delta E3$, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 7) inserted in E1.
16	57-61	The claims are directed to a method of making and harvesting of a recombinant adenoviral particle that contains a gene encoding an HIV Pol protein.
17	62, 65, 66	The claim is directed to a method of generating a cellular mediated immune response to HIV Pol protein with the recombinant adenoviral particle.
18	63, 64	The claim is directed to a method of generating a cellular mediated immune response to HIV Pol protein with the recombinant adenoviral particle in addition to administering a DNA plasmid vaccine.
19	67-70, 72, 73, 75	The claims are directed to an adenoviral vector that is at least partially deleted of ΔΕ1, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 9) inserted in the parallel orientation of Ε1.
20	67-70, 72, 73, 75	The claims are directed to an adenoviral vector that is at least partially deleted of <u>AE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 11) inserted in the parallel orientation of E1.
21	67-70, 72, 73, 75	The claims are directed to an adenoviral vector that is at least partially deleted of ΔΕ1, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 13) inserted in the parallel orientation of E1.
22	67-70, 72, 73, 75	The claims are directed to an adenoviral vector that is at least partially deleted of <u>AEI</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 15) inserted in the parallel orientation of E1.
23	71	The claim is directed to an adenoviral vector that is at least partially deleted of <u>AE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Nef protein (SEQ ID NO: 9)</u> inserted in the antiparallel orientation of E1.
24	71	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 11) inserted in the antiparallel orientation of E1.
25	71	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 13) inserted in the antiparallel orientation of E1.
26	71	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 15) inserted in the antiparallel orientation of E1.
27	74	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ and $\Delta E3$, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 9) inserted in E1.
28	74	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ and $\Delta E3$, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 11) inserted in E1.
29	74	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ and $\Delta E3$, the vector contains the cis-acting packaging sequence of the wild type

International application No.

PCT/US01/28861

		THE STATE OF THE S
		adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 13)
		inserted in E1.
30	74	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$
		and $\Delta E3$, the vector contains the cis-acting packaging sequence of the wild type
		adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 15)
		inserted in E1.
31	76-80	The claims are directed to a method of making and harvesting of a recombinant
		adenoviral particle that contains a gene encoding an HIV Nef protein.
32	81, 84, 85	The claims are directed to a method of generating a cellular mediated immune
		response to HIV Nef with the recombinant adenoviral particle.
33	82, 83	The claims are directed to a method of generating a cellular mediated immune
		response to HIV Nef with the recombinant adenoviral particle in addition to
		administering a DNA plasmid vaccine.
34	86a	The claim is drawn to a multivalent vaccine wherein gag, pol and nef are expressed
		from three individual vectors.
35	86b, 88, 89	The claims are drawn to a multivalent vaccine wherein gag, pol and nef are expressed
		from one individual vectors.
36	86c, 88	The claims are drawn to a multivalent vaccine wherein gag, pol and nef are expressed
		from two individual vectors, one expressing nef-pol fusion and one expressing gag.
37	86d, 87, 88	The claims are drawn to a multivalent vaccine wherein gag, pol and nef are expressed
•	1	from two individual vectors, one expressing gag-pol fusion and one expressing nef.
38	86e, 88	The claims are drawn to a multivalent vaccine wherein gag, pol and nef are expressed
	1	from two individual vectors, one expressing nef-gag fusion and one expressing pol.
39	86f, 88	The claims are drawn to a multivalent vaccine wherein gag, pol and nef are expressed
	1 '	from a single vectors as a fusion protein.
40	86g, 88	The claims are drawn to a multivalent vaccine wherein gag and pol are expressed
	3, 33	from two individual vectors.
41	86h, 88, 89	The claims are drawn to a multivalent vaccine wherein gag and pol are expressed
•	1 00,00,00	individually from one vector.
42	86i, 88	The claims are drawn to a multivalent vaccine wherein pol and nef are expressed
74	00., 00	from two individual vectors.
43	86j, 88, 89	The claims are drawn to a multivalent vaccine wherein pol and nef are expressed
43	00], 00, 05	from individually from one vector.
44	86k, 88	The claims are drawn to a multivalent vaccine wherein nef and gag are expressed
~	00x, 00	individually from one vector.
45	861, 88, 89	The claims are drawn to a multivalent vaccine wherein nef and gag are expressed
43	001, 00, 03	individually from one vector.
46	86m, 88	The claims are drawn to a multivalent vaccine wherein gag and pol are expressed as a
70	SUIL, 86	fusion protein from one vector.
47	86n, 88	The claims are drawn to a multivalent vaccine wherein pol and nef are expressed as a
4/	0011, 00	fusion protein from one vector.
40	965 99	The claims are drawn to a multivalent vaccine wherein nef and gag are expressed as a
48	86o, 88	
		fusion protein from one vector.

The inventions listed as Groups 1-48 do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The technical feature linking groups 1-33 appears to be a recombinant adenoviral vector wherein the adenoviral vector is at least partially deleted in E1 but the vector may contain more deletions, the vector contains wild type sequences including packaging signals and a gene encoding a heterologous HIV protein or fragments thereof. End et al. (WO 96/39178) disclose a recombinant adenoviral vector that is deleted in E1 and partially deleted in E3, the remainder of the adenoviral vector contains wild type sequences. The vector additionally contains an insertion of a heterologous protein which includes HIV proteins (see abstract and claims 1 and 5). Therefore, the technical feature linking the inventions of groups 1-45 does not constitute a special technical feature as defined by PCT Rule 13.2, as it does not define a contribution over the prior art.

The special technical feature of the following groups 1-3, 7-15, 19-30 and 34-48 is considered to be the combination of sequences that is disclosed in each group, see individual claim groupings above for the different sequences. The DNA disclosed in each group is made up of a different sequence having a different structure and different function.

International application No.

PCT/US01/28861

The special technical feature of group 4, 16 and 31 is considered to be a method of producing recombinant adenoviral particles. Each group contains different sequences hence the resulting particles would have different structures and functions associated with the particle.

The special technical feature of group 5, 17 and 32 is considered to be a method of producing a cellular mediated immune response to the heterologous protein encoded by the different adenoviral vectors. Each group contains different sequences a encoding different protein, therefore the resulting immune response will also be different.

The special technical feature of group 6, 18 and 33 is considered to be a method of producing a cellular mediated immune response to the heterologous protein encoded by the different adenoviral vectors in conjunction with immunizing the individual a DNA plasmid vaccine. Each method contains different sequences encoding a different protein, therefore the resulting immune response will also be different.

Accordingly, groups 1-48 are not so linked by the same or corresponding technical feature as to form a single general inventive concept.

Continuation of B. FIELDS SEARCHED Item 3:

WEST 2.0, STN-BIOSIS, MEDLINE adenoviral vector, deletion, HIV, Gag, polyadenylation signal, CMV promoter

(19) World Intellectual Property Organization International Bureau



1 10118 BIII 1011 I BIBIR OOTO 1011 I HI HA HAR KAN OOTO 1011 OO OO OO OO OO

(43) International Publication Date 21 March 2002 (21.03.2002)

PCT

(10) International Publication Number WO 02/22080 A3

(51) International Patent Classification7:

C12N 15/86

(21) International Application Number: PCT/US01/28861

(22) International Filing Date:

14 September 2001 (14.09.2001)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data: 60/233,180 15 September 2000 (15.09.2000) U

- (71) Applicant (for all designated States except US): MERCK & CO., INC. [US/US]: 126 East Lincoln Avenue. Rahway. NJ 07065-0907 (US).
- (72) Inventors; and
- (75) Inventors/Applicants (for US only): EMINI, Emilio, A. [US/US]: 126 East Lincoln Avenue. Rahway. NJ 07065-0907 (US). YOUIL, Rima [AU/US]: 126 East Lincoln Avenue. Rahway. NJ 07065-0907 (US). BETT, Andrew, J. [CA/US]: 126 East Lincoln Avenue. Rahway. NJ 07065-0907 (US). CHEN, Ling [US/US]: 126 East Lincoln Avenue. Rahway. NJ 07065-0907 (US). KASLOW, David, C. [US/US]: 126 East Lincoln Avenue, Rahway. NJ 07065-0907 (US). SHIVER, John, W. [US/US]: 126 East Lincoln Avenue. Rahway. NJ 07065-0907 (US). TONER, Timothy, J. [US/US]: 126 East Lincoln Avenue. Rahway. NJ 07065-0907 (US). CASIMIRO, Daniel, R. [PH/US]: 126 East Lincoln Avenue. Rahway. NJ 07065-0907 (US).

- (74) Common Representative: MERCK & CO., INC.: 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US).
- (81) Designated States (national): AE. AG. AL. AM. AT. AU. AZ. BA, BB, BG. BR. BY. BZ. CA. CH. CN. CO. CR. CU. CZ. DE, DK. DM, DZ. EC. EE. ES. FI. GB. GD. GE. GH. GM. HR. HU, ID. IL. IN. IS. JP. KE. KG. KR, KZ. LC, LK. LR. LS. LT. LU. LV. MA. MD. MG, MK. MN. MW, MX, MZ. NO, NZ. PH. PL. PT. RO. RU. SD. SE. SG. SI. SK. SL. TJ. TM. TR. TT. TZ. UA, UG. US. UZ. VN. YU. ZA. ZW.
- (84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW). Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM). European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR). OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

- with international search report
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments
- (88) Date of publication of the international search report: 2 May 2002

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: ENHANCED FIRST GENERATION ADENOVIRUS VACCINES EXPRESSING CODON OPTIMIZED HIVI-GAG. POL. NEF AND MODIFICATIONS

hanced stability and growth properties and greater cellular-mediated immunity are described within this specification. These adenoviral vectors are utilized to generate and produce through cell culture various adenoviral-based HIV-1 vaccines which contain HIV-1 gag. HIV-1 pol and/or HIV-1 nef polynucleotide pharmaceutical products, and biologically relevant modifications thereof. These adenovirus vaccines, when directly introduced into living vertebrate tissue, preferably a mammalian host such as a human or a non-human mammal of commercial or domestic veterinary importance, express the HIV1- Gag, Pol and/or Nef protein or biologically modification thereof, inducing a cellular immune response which specifically recognizes HIV-1. The exemplified polynucleotides of the present invention are synthetic DNA molecules encoding HIV-1 Gag, encoding codon optimized HIV-1 Pol (including constructs wherein protease, reverse transcriptase, RNAse H and integrase activity of HIV-1 Pol is inactivated). HIV-1 Nef and derivatives of optimized HIV-1 Nef, including nef mutants which effect wild type characteristics of Nef, such as myristylation and down regulation of host CD4. The adenoviral vaccines of the present invention, when administered alone or in a combined modality regime, will offer a prophylactic advantage to previously uninfected individuals and/or provide a therapeutic effect by reducing viral load levels within an infected individual, thus prolonging the asymptomatic phase of HIV-1 infection.



International application No.

IPC(7) US CL According to	SIFICATION OF SUBJECT MATTER : C12N 15/86 : 435/456 International Patent Classification (IPC) or to both n	ational classification and IPC	
B. FIEL	DS SEARCHED		
U.S. : 42	cumentation searched (classification system followed 24/205.1, 207.1, 227.1, 233.1; 435/69.1, 69.3, 173.3	3, 235.1, 320.1, 430; 330/23.72;	
	on searched other than minimum documentation to the		
Electronic da Please See C	ta base consulted during the international search (namontinuation Sheet	ne of data base and, where practicable, s	earch terms used)
C. DOC	UMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where ap	propriate, of the relevant passages	Relevant to claim No.
X	WO 96/39178 (ERTL et al.) 12 December 1996 (12 and claims 1 and 5.	.12.1996), see page 5, 6,10, 12, 13	1-3, 8-11, 18
Y			4, 5, 13-17, 29, 30, 32, 34, 35, 37
x	US 6,019,978 A (ERTL et al.) 1 February 2000 (01	/02/2000), see columns 2, 7 and 8.	1-3, 8-11, 18
Y			4, 5, 13-17, 29, 30, 32, 34, 35, 37
X,P	US 6,287,571 A A (ERTL et al.) 11 September 200	1 (11/09/2001), see columns 2, 7, 8	1, 9, 18
x	and claim 1. US 5,643,579A (HUNG et al.) 1 July 1997 (01/07/)	1997), see examples 1, 2, 25 and 26.	1-3, 8, 9-11, 18
Y			4,5,13-17, 29, 30, 32, 34, 35, 37
Y	WANG et al. The use of an E1-deleted, replication expressing the rabies virus glycoprotein for early va Journal of Virology (March 1997) Vol. 71, No. 5, p	accination of mice against rabies virus.	1-3, 9-11, 13-18
Purthe	r documents are listed in the continuation of Box C.	See patent family annex.	L
_	Special categories of cited documents:	"T" later document published after the inte	
"A" documen	t defining the general state of the art which is not considered to be	date and not in conflict with the appli principle or theory underlying the inv	ention
	pplication or patent published on or after the international filing date	"X" document of particular relevance; the considered novel or cannot be conside when the document is taken alone	
establish specified		"Y" document of particular relevance; the considered to involve an inventive ste combined with one or more other sue	p when the document is h documents, such combination
"O" documen	nt referring to an oral disclosure, use, exhibition or other means	being obvious to a person skilled in the	ne art
	n published prior to the international filing date but later than the date claimed	"&" document member of the same patent	
	actual completion of the international search	Date of mailing of the international ser	arch report
06 February	2002 (06.02.2002)	Authorized officer	
Co	nailing address of the ISA/US mmissioner of Patents and Trademarks	Ulrike Winkler, Ph.D.	1/100
	x PCT sshington, D.C. 20231	Marie	Collins - 12
1 '	lo. (703)305-3230	Telephone No. 703-308-0196	<u> </u>
Form PCT/IS	SA/210 (second sheet) (July 1998)		1

International application No.

PCT/US01/28861

INTERNATIONAL SEARCH REPORT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
Y	NATUK et al. Immunogenicity of recombinant human adenovirus -human immunodeficiency virus vaccines in chimpanzees. Aids Research and Human Retroviruses (1993) Vol. 9, No. 5, pp395-404, see material and methods.	1, 9, 29, 30, 32
Y	PREVEC et al. Immune response to HIV-1 gag antigens induced by recombinant adenovirus vectors in mice and rhesus macaque monkeys. Journal of Acquired Immune Deficincy Syndrome. (1991) Vol. 4, No. 6 pp. 568-76, see abstract.	1, 9, 29, 30, 32
Y	LORI et al. Rapid protection against human immunodeficiency virus type 1 (HIV-1) replication mediated by high efficiency non-retroviral delivery of genes interfering with HIV-1 tat and gag. Gene Therapy (1994) Vol. 1, No. 1, pp. 27-31, see abstract.	1, 9
Y	PFARR et al. Differential effects of polyadenylation regions on gene expression in maximalian cells. DNA (1986) Vol. 5, No. 2, pp.115-22, see abstract.	. 16
Y	NATUK et al. Adenovirus vectored vaccine. Developmental Biological Standards (1994) Vol. 82, pp. 71-77, see abstract.	1, 9
	·	

International application No.

PCT/US01/28861

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)				
This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:				
1. Claim Nos.: because they relate to subject matter not required to be searched by this Authority, namely:				
2. Claim Nos.: 31 because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically: This claim could not be searched because applicant did not provide a CRF.				
Claim Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).				
Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)				
This International Searching Authority found multiple inventions in this international application, as follows: Please See Continuation Sheet				
As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.				
2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.				
3. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:				
4. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-5, 8-11, 13-18, 29-32, 34, 35, 37				
Remark on Protest				
No protest accompanied the payment of additional search fees.				

Form PCT/ISA/210 (continuation of first sheet(1)) (July 1998)

International application No.

PCT/US01/28861

The special technical feature of group 4, 16 and 31 is considered to be a method of producing recombinant adenoviral particles. Each group contains different sequences hence the resulting particles would have different structures and functions associated with the particle.

The special technical feature of group 5, 17 and 32 is considered to be a method of producing a cellular mediated immune response to the heterologous protein encoded by the different adenoviral vectors. Each group contains different sequences a encoding different protein, therefore the resulting immune response will also be different.

The special technical feature of group 6, 18 and 33 is considered to be a method of producing a cellular mediated immune response to the heterologous protein encoded by the different adenoviral vectors in conjunction with immunizing the individual a DNA plasmid vaccine. Each method contains different sequences encoding a different protein, therefore the resulting immune response will also be different.

Accordingly, groups 1-48 are not so linked by the same or corresponding technical feature as to form a single general inventive concept.

Continuation of B. FIELDS SEARCHED Item 3:

WEST 2.0, STN-BIOSIS, MEDLINE adenoviral vector, deletion, HIV, Gag, polyadenylation signal, CMV promoter

International application No.

	1	and ΔE3, the vector contains the cis-acting packaging sequence of the wild type
		adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 1)
		inserted in E1.
14	55	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$
		and ΔE3, the vector contains the cis-acting packaging sequence of the wild type
		adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 5)
		inserted in E1.
15	55	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$
		and ΔE3, the vector contains the cis-acting packaging sequence of the wild type
		adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 7)
		inserted in Et.
16	57-61	The claims are directed to a method of making and harvesting of a recombinant
		adenoviral particle that contains a gene encoding an HIV Pol protein.
17	62, 65, 66	The claim is directed to a method of generating a cellular mediated immune response
		to HIV Pol protein with the recombinant adenoviral particle.
18	63, 64	The claim is directed to a method of generating a cellular mediated immune response
	100,00	to HIV Pol protein with the recombinant adenoviral particle in addition to
		administering a DNA plasmid vaccine.
19	67-70, 72,	The claims are directed to an adenoviral vector that is at least partially deleted of
17		ΔE1, the vector contains the cis-acting packaging sequence of the wild type
	73, 75	adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 9)
		inserted in the parallel orientation of Et.
	67.70.72	The claims are directed to an adenoviral vector that is at least partially deleted of
20	67-70, 72,	The claims are directed to an adenoviral vector that is at least partially defected of
	73, 75	ΔΕ1, the vector contains the cis-acting packaging sequence of the wild type
	Į.	adenovirus genome, and a gene which encodes an HIV Ncf protein (SEQ ID NO: 11)
		inserted in the parallel orientation of E1.
21	67-70, 72,	The claims are directed to an adenoviral vector that is at least partially deleted of
	73, 75	$\Delta E1$, the vector contains the cis-acting packaging sequence of the wild type
		adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 13)
		inserted in the parallel orientation of E1.
22	67-70, 72,	The claims are directed to an adenoviral vector that is at least partially deleted of
	73, 75	ΔE1, the vector contains the cis-acting packaging sequence of the wild type
		adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 15)
	1	inserted in the parallel orientation of E1.
23	71	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$,
	\ ''	the vector contains the cis-acting packaging sequence of the wild type adenovirus
		genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 9) inserted in
		the antiparallel orientation of E1.
24	71	The claim is directed to an adenoviral vector that is at least partially deleted of ΔE_1 ,
£ -) "1	the vector contains the cis-acting packaging sequence of the wild type adenovirus
	ļ	genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 11) inserted in
	İ	the antiparallel orientation of E1.
26	 	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E I$,
25	71	the vector contains the cis-acting packaging sequence of the wild type adenovirus
		genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 13) inserted in
		the antiparallel orientation of E1.
26	71	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$.
		the vector contains the cis-acting packaging sequence of the wild type adenovirus
		genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 15) inserted in
		the antiparallel orientation of E1.
27	74	The claim is directed to an adenoviral vector that is at least partially deleted of ΔEI
		and $\Delta E3$, the vector contains the cis-acting packaging sequence of the wild type
		adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 9)
		inserted in E1.
28	74	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$
40	' ⁻	and $\Delta E3$, the vector contains the cis-acting packaging sequence of the wild type
		adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 11)
	l	
		inserted in E1.
29	74	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$
67	1 ' '	and Δ E3, the vector contains the cis-acting packaging sequence of the wild type

International application No.

		the principle of the principle type
	1	and ΔE3, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 1)
		inserted in E1. The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$
14	55	and $\Delta E3$, the vector contains the cis-acting packaging sequence of the wild type
	ŀ	adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 5)
	İ	inserted in E1.
15	55	The claim is directed to an adenoviral vector that is at least partially deleted of ΔΕ1
13	1 33	and AE3, the vector contains the cis-acting packaging sequence of the wild type
	ı	adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 7)
	ı	inserted in E1.
16	57-61	The claims are directed to a method of making and harvesting of a recombinant
	15.5	adenoviral particle that contains a gene encoding an HIV Pol protein.
17	62, 65, 66	The claim is directed to a method of generating a cellular mediated immune response
••	52, 52, 53	to HIV Pol protein with the recombinant adenoviral particle.
18	63, 64	The claim is directed to a method of generating a cellular mediated immune response
	· ·	to HIV Pol protein with the recombinant adenoviral particle in addition to
	:	administering a DNA plasmid vaccine.
19	67-70, 72,	The claims are directed to an adenoviral vector that is at least partially deleted of
	73, 75	ΔΕ1, the vector contains the cis-acting packaging sequence of the wild type
		adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 9)
		inserted in the parallel orientation of E1.
20	67-70, 72,	The claims are directed to an adenoviral vector that is at least partially deleted of
	73, 75	ΔΕ1, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 11)
		inserted in the parallel orientation of E1.
	60.00.00	The claims are directed to an adenoviral vector that is at least partially deleted of
21	67-70, 72,	ΔE1, the vector contains the cis-acting packaging sequence of the wild type
	73, 75	adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 13)
		inserted in the parallel orientation of E1.
22	67-70, 72,	The claims are directed to an adenoviral vector that is at least partially deleted of
22	73, 75	AF1, the vector contains the cis-acting packaging sequence of the wild type
	,3, ,3	adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 15)
	1	inserted in the parallel orientation of E1.
23	71	The claim is directed to an adenoviral vector that is at least partially deleted of ΔE_1 ,
	ļ	the vector contains the cis-acting packaging sequence of the wild type adenovirus
		genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 9) inserted in
		the antiparallel orientation of E1.
24	71	The claim is directed to an adenoviral vector that is at least partially deleted of ΔE_1 ,
		the vector contains the cis-acting packaging sequence of the wild type adenovirus
	ĺ	genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 11) inserted in
		the antiparallel orientation of E1. The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$,
25	71	the vector contains the cis-acting packaging sequence of the wild type adenovirus
i	1	genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 13) inserted in
ŀ		the antiparallel orientation of E1.
26	71	The claim is directed to an adenoviral vector that is at least partially deleted of ΔE_1 .
20	1"	the vector contains the cis-acting packaging sequence of the wild type adenovirus
Ì		genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 15) inserted in
		the antiparallel orientation of E1.
27	74	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$
-		and AE3, the vector contains the cis-acting packaging sequence of the wild type
ł		adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 9)
		inserted in E1.
28	74	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$
		and $\Delta E3$, the vector contains the cis-acting packaging sequence of the wild type
		adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 11)
		inserted in E1.
29	74	The claim is directed to an adenoviral vector that is at least partially deleted of ΔE_1
		and $\Delta E3$, the vector contains the cis-acting packaging sequence of the wild type
_		and about (fully 1008)

International application No.

PCT/US01/28861

		adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 13)
	T	adenovirus genome, and a gene which encodes an inviter probations of
		inserted in E1.
30	74	The claim is directed to an adenoviral vector that is at least partially deleted of ΔE_1
30	1	and $\Delta E3$, the vector contains the cis-acting packaging sequence of the wild type
	ļ	adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 15)
		inserted in E1.
	76-80	The claims are directed to a method of making and harvesting of a recombinant
31	70-00	adenoviral particle that contains a gene encoding an HIV Nef protein.
	81, 84, 85	The claims are directed to a method of generating a cellular mediated immune
32	81, 04, 55	response to HIV Nef with the recombinant adenoviral particle.
	92 93	The claims are directed to a method of generating a cellular mediated immune
33	82, 83	response to HIV Nef with the recombinant adenoviral particle in addition to
		administering a DNA plasmid vaccine.
		The claim is drawn to a multivalent vaccine wherein gag, pol and nef are expressed
34	86a	from three individual vectors.
		The claims are drawn to a multivalent vaccine wherein gag, pol and nef are expressed
35	86b, 88, 89	
		from one individual vectors. The claims are drawn to a multivalent vaccine wherein gag, pol and nef are expressed
36	86c, 88	The claims are drawn to a multivatelli vaccile wherein gag, por and me expressing and
50		from two individual vectors, one expressing nef-pol fusion and one expressing gag.
37	86d, 87, 88	The claims are drawn to a multivalent vaccine wherein gag, pol and nef are expressed
31	,	from two individual vectors, one expressing gag-pol fusion and one expressing nef.
20	86e, 88	The claims are drawn to a multivalent vaccine wherein gag, pol and nef are expressed
38	000, 00	from two individual vectors, one expressing nef-gag fusion and one expressing pol.
	86f, 88	The claims are drawn to a multivalent vaccine wherein gag, pol and nef are expressed
39	001, 00	from a single vectors as a fusion protein.
	86g, 88	The claims are drawn to a multivalent vaccine wherein gag and pol are expressed
40	80g, 80	from two individual vectors.
	86h, 88, 89	The claims are drawn to a multivalent vaccine wherein gag and pol are expressed
41	800, 80, 69	individually from one vector.
		The claims are drawn to a multivalent vaccine wherein pol and nef are expressed
42	86i, 88	from two individual vectors.
		The claims are drawn to a multivalent vaccine wherein pol and nef are expressed
43	86j, 88, 89	from individually from one vector.
	<u> </u>	The claims are drawn to a multivalent vaccine wherein nef and gag are expressed
44	86k, 88	
		individually from one vector.
45	861, 88, 89	The claims are drawn to a multivalent vaccine wherein nef and gag are expressed
		individually from one vector.
46	86m, 88	The claims are drawn to a multivalent vaccine wherein gag and pol are expressed as a
-10	J 33.27, 22	fusion protein from one vector.
47	86n, 88	The claims are drawn to a multivalent vaccine wherein pol and nef are expressed as a
"′	9011, 00	fusion protein from one vector.
10		The claims are drawn to a multivalent vaccine wherein nef and gag are expressed as a
48	86o, 88	fusion protein from one vector.
		Itasion protess from our version

The inventions listed as Groups 1-48 do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The technical feature linking groups 1-33 appears to be a recombinant adenoviral vector wherein the adenoviral vector is at least partially deleted in E1 but the vector may contain more deletions, the vector contains wild type sequences including packaging signals and a gene encoding a heterologous HIV protein or fragments thereof. Erd et al. (WO 96/39178) disclose a recombinant adenoviral vector that is deleted in E1 and partially deleted in E3, the remainder of the adenoviral vector contains wild type sequences. The vector additionally contains an insertion of a heterologous protein which includes HIV proteins (see abstract and claims 1 and 5). Therefore, the technical feature linking the inventions of groups 1-45 does not constitute a special technical feature as defined by PCT Rule 13.2, as it does not define a contribution over the prior art.

The special technical feature of the following groups 1-3, 7-15, 19-30 and 34-48 is considered to be the combination of sequences that is disclosed in each group, see individual claim groupings above for the different sequences. The DNA disclosed in each group is made up of a different sequence having a different structure and different function.

REVISED VERSION

(19) World Intellectual Property Organization International Bureau





(43) International Publication Date 21 March 2002 (21.03.2002)

PCT

(10) International Publication Number WO 02/022080 A3

(51) International Patent Classification7: C12N 15/86

(21) International Application Number: PCT/US01/28861

(22) International Filing Date:

14 September 2001 (14.09.2001)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

60/233,180 15 September 2000 (15.09.2000)

2000) US

- (71) Applicant (for all designated States except US): MERCK & CO., INC. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US).
- (72) Inventors; and
- (75) Inventors/Applicants (for US only): EMINI, Emilio, A. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). YOUIL, Rima [AU/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). BETT, Andrew, J. [CA/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). CHEN, Ling [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). KASLOW, David, C. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). SHIVER, John, W. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). TONER, Timothy, J. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). CASIMIRO, Daniel, R. [PH/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US).
- (74) Common Representative: MERCK & CO., INC.; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US).

- (81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.
- (84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

- with international search report
- (88) Date of publication of the international search report: 2 May 2002

Date of publication of the revised international search report: 16 January 2003

(15) Information about Corrections:

see PCT Gazette No. 03/2003 of 16 January 2003, Section II

Previous Correction:

see PCT Gazette No. 30/2002 of 25 July 2002, Section II

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: ENHANCED FIRST GENERATION ADENOVIRUS VACCINES EXPRESSING CODON OPTIMIZED HIV1-GAG, POL, NEF AND MODIFICATIONS

(57) Abstract: First generation adenoviral vectors and associated recombinant adenovirus-based HTV vaccines which show enhanced stability and growth properties and greater cellular-mediated immunity are described within this specification. These adenoviral vectors are utilized to generate and produce through cell culture various adenoviral-based HTV-1 vaccines which contain HTV-1 gag, HTV-1 pol and/or HTV-1 nef polynucleotide pharmaceutical products, and biologically relevant modifications thereof. These adenovirus vaccines, when directly introduced into living vertebrate tissue, preferably a mammalian host such as a human or a non-human mammal of commercial or domestic veterinary importance, express the HTV-1 Gag, Pol and/or Nef protein or biologically modification thereof, inducing a cellular immune response which specifically recognizes HTV-1. The exemplified polynucleotides of the present invention are synthetic DNA molecules encoding HTV-1 Gag, encoding codon optimized HTV-1 Pol, derivatives of optimized HTV-1 Pol (including constructs wherein protease, reverse transcriptase, RNAse H and integrase activity of HTV-1 Pol is inactivated), HTV-1 Nef and derivatives of optimized HTV-1 Nef, including nef mutants which effect wild type characteristics of Nef, such as myristylation and down regulation of host CD4. The adenoviral vaccines of the present invention, when administered alone or in a combined modality regime, will offer a prophylactic advantage to previously uninfected individuals and/or provide a therapeutic effect by reducing viral load levels within an infected individual, thus prolonging the asymptomatic phase of HTV-1 infection.



International application No.

PCT/US01/28861

IPC(7) US CL According B. FIE	ASSIFICATION OF SUBJECT MATTER : C12N 15/86 : 435/456 to International Patent Classification (IPC) or to both a LDS SEARCHED documentation searched (classification system followed 424/205.1, 207.1, 227.1, 233.1; 435/69.1, 69.3, 173.	by classification symbols)		
	tion searched other than minimum documentation to th		d in the fields searched	
Electronic of Please See	data base consulted during the international search (na Continuation Sheet	me of data base and, where practicable, s	earch terms used)	
C. DO	CUMENTS CONSIDERED TO BE RELEVANT			
Category *	Citation of document, with indication, where a	ppropriate, of the relevant passages	Relevant to claim No.	
Х	WO 96/39178 (ERTL et al.) 12 December 1996 (12		1-3, 8-11, 18	
Ÿ	and claims 1 and 5.		4, 5, 13-17, 29-32, 34, 35, 37	
x	US 6,019,978 A (ERTL et al.) 1 February 2000, (01/02/2000), see columns 2, 7 and 8. 1-3, 8-11, 18			
Ÿ			4, 5, 13-17, 29-32, 34, 35, 37	
X,P	US 6,287,571 B (ERTL et al.) 11 September 200 and claim 1.	01 (11/09/2001), see columns 2, 7, 8	1, 9, 18	
x	US 5,643,579A (HUNG et al.) 1 July 1997 (01/07/	1997), see examples 1, 2, 25 and 26.	1-3, 8, 9-11, 18	
Y			4,5,13-17, 29-32, 34, 35, 37	
Y	WANG et al. The use of an E1-deleted, replication expressing the rabies virus glycoprotein for early vi Journal of Virology (March 1997) Vol. 71, No. 5, 1	accination of mice against rabies virus.	1-3, 9-11, 13-18	
Purths	er documents are listed in the continuation of Box C.	See patent family annex.		
•	Special categories of cited documents:	T later document published after the in		
"A" docume be of pa	nt defining the general state of the art which is not considered to rticular relevance	priority date and not in conflict with understand the principle or theory un	derlying the invention	
"E" carlier a	application or patent published on or after the international filing	"X" document of particular relevance; the considered novel or cannot be considered novel or cannot be considered novel or cannot be considered.	kred to involve an inventive	
to estab	document which may throw doubts on priority claim(s) or which is cited "Y" o establish the publication date of another citation or other special reason as specified) document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such			
"O" docume	combination being obvious to a person skilled in the art			
	"&" document member of the same patent family when published prior to the international filing date but later than the write date claimed			
Date of the actual completion of the international search Date of mailing of the international search report 19 AUG 2002				
06 February 2002 (06.02.2002) Name and mailing address of the ISA/US Authorized officer				
	Commissioner of Patents and Trademarks			
Во	x PCT shington, D.C. 20231	Ulrike Winkler, Ph.D.	7	
	Facsimile No. (703)305-3230 Telephone No. 703-308-0196			
- DOTAG	14/010 (assert 1 - 1) (T 1 - 1000)	L		

Form PCT/ISA/210 (second sheet) (July 1998)

International application No.

PCT/US01/28861

INTERNATIONAL SEARCH REPORT

C. (Contin	uation) DOCUMENTS CONSIDERED TO BE RELEVANT	
Category •	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	NATUK et al. Immunogenicity of recombinant human adenovirus -human immunodeficiency virus vaccines in chimpanzees. Aids Research and Human Retroviruses (1993) Vol. 9, No. 5, pp395-404, see material and methods.	1, 9, 29-32
Y	PREVEC et al. Immune response to HIV-1 gag antigens induced by recombinant adenovirus vectors in mice and rhesus macaque monkeys. Journal of Acquired Immune Deficincy Syndrome. (1991) Vol. 4, No. 6 pp. 568-76, see abstract.	1, 9, 29-32
Y	LORI et al. Rapid protection against human immunodeficiency virus type 1 (HIV-1) replication mediated by high efficiency non-retroviral delivery of genes interfering with HIV-1 tat and gag. Gene Therapy (1994) Vol. 1, No. 1, pp. 27-31, see abstract.	1,9
Y	PFARR et al. Differential effects of polyadenylation regions on gene expression in mammalian cells. DNA (1986) Vol. 5, No. 2, pp.115-22, see abstract.	16
Y	NATUK et al. Adenovirus vectored vaccine. Developmental Biological Standards (1994) Vol. 82, pp. 71-77, see abstract.	1,9
I		
		į.
		<u> </u>

International application No.

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)				
This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:				
Claim Nos.: because they relate to subject matter not required to be searched by this Authority, namely:				
2. Claim Nos.: because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:				
3. Claim Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).				
Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)				
This International Searching Authority found multiple inventions in this international application, as follows: Please See Continuation Sheet				
 As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.: 				
4. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-5, 8-11, 13-18, 29-32, 34, 35, 37				
Remark on Protest The additional search fees were accompanied by the applicant's protest. No protest accompanied the payment of additional search fees.				

International application No.

PCT/US01/28861

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group	Claims	
1	1-5, 8-11, 13-18, 29, 30, 31, 32, 34, 35, 37	The claims are directed to an adenoviral vector that is at least partially deleted of ΔEI , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Gag protein (SEQ ID NO: 29) inserted in the parallel orientation of E1. In addition the vector contains a promoter and a polyadenylation signal.
2	6, 7, 36	The claims are directed to an adenoviral vector that is at least partially deleted of <u>AE1</u> and <u>AE3</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Gag protein (SEQ ID NO: 29).
3	12, 33	The claims are directed to an adenoviral vector that is at least partially deleted of $\Delta E1$, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV protein inserted in the antiparallel orientation of E1.
4	19-23, 38-42	The claims are directed to a method of making and harvesting of a recombinant adenoviral particle that contains a gene encoding an HIV Gag protein.
5	24, 27, 28, 43, 46, 47	The claim is directed to a method of generating a cellular mediated immune response to HIV Gag protein with the recombinant adenoviral particle.
6	25, 26, 44, 45	The claim is directed to a method of generating a cellular mediated immune response to HIV Gag protein with the recombinant adenoviral particle in addition to administering a DNA plasmid vaccine.
7	48-51, 53, 54, 56	The claims are directed to an adenoviral vector that is at least partially deleted of <u>AE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Pol protein (SEQ ID NO: 1)</u> inserted in the parallel orientation of E1.
8	48-51, 53, 54, 56	The claims are directed to an adenoviral vector that is at least partially deleted of <u>AE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Pol protein (SEQ ID NO: 5)</u> inserted in the parallel orientation of E1.
9	48-51, 53, 54, 56	The claims are directed to an adenoviral vector that is at least partially deleted of <u>AE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Pol protein (SEQ ID NO: 7)</u> inserted in the parallel orientation of E1.
10	52	The claim is directed to an adenoviral vector that is at least partially deleted of ΔEI , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 1) inserted in the antiparallel orientation of E1.
11	52	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 5) inserted in the antiparallel orientation of E1.
12	52	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 7) inserted in the antiparallel orientation of E1.
13	55	The claim is directed to an adenoviral vector that is at least partially deleted of AE1

Form PCT/ISA/210 (second sheet) (July 1998)

International application No.

		and $\Delta E3$, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 1) inserted in E1.
14	55	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ and $\Delta E3$, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 5) inserted in E1.
15	55	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ and $\Delta E3$, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 7) inserted in $E1$.
16	57-61	The claims are directed to a method of making and harvesting of a recombinant adenoviral particle that contains a gene encoding an HIV Pol protein.
17	62, 65, 66	The claim is directed to a method of generating a cellular mediated immune response to HIV Pol protein with the recombinant adenoviral particle.
18	63, 64	The claim is directed to a method of generating a cellular mediated immune response to HIV Pol protein with the recombinant adenoviral particle in addition to administering a DNA plasmid vaccine.
19	67-70, 72, 73, 75	The claims are directed to an adenoviral vector that is at least partially deleted of <u>AE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Nef protein (SEQ ID NO: 9)</u> inserted in the parallel orientation of E1.
20	67-70, 72, 73, 75	The claims are directed to an adenoviral vector that is at least partially deleted of $\Delta E1$, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 11) inserted in the parallel orientation of E1.
21	67-70, 72, 73, 75	The claims are directed to an adenoviral vector that is at least partially deleted of <u>AEI</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Nef protein (SEQ ID NO: 13)</u> inserted in the parallel orientation of E1.
22	67-70, 72, 73, 75	The claims are directed to an adenoviral vector that is at least partially deleted of <u>AEI</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Nef protein (SEQ ID NO: 15)</u> inserted in the parallel orientation of E1.
23	71	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 9) inserted in the antiparallel orientation of E1.
24	71	The claim is directed to an adenoviral vector that is at least partially deleted of ΔEI , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 11) inserted in the antiparallel orientation of E1.
25	71	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 13) inserted in the antiparallel orientation of E1.
26	71	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 15) inserted in the antiparallel orientation of E1.
27	74	The claim is directed to an adenoviral vector that is at least partially deleted of ΔE_1 and ΔE_3 , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 9) inserted in E1.
28	74	The claim is directed to an adenoviral vector that is at least partially deleted of ΔE_1 and ΔE_3 , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 11)
		inserted in E1.

International application No.

PCT/US01/28861

		adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 13)
	}	inserted in E1.
30	74	
30	/4	The claim is directed to an adenoviral vector that is at least partially deleted of ΔE_1
	i	and AE3, the vector contains the cis-acting packaging sequence of the wild type
		adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 15)
		inserted in E1.
31	76-80	The claims are directed to a method of making and harvesting of a recombinant
		adenoviral particle that contains a gene encoding an HIV Nef protein.
32	81, 84, 85	The claims are directed to a method of generating a cellular mediated immune
		response to HIV Nef with the recombinant adenoviral particle.
33	82, 83	The claims are directed to a method of generating a cellular mediated immune
		response to HIV Nef with the recombinant adenoviral particle in addition to
		administering a DNA plasmid vaccine.
34	86a	The claim is drawn to a multivalent vaccine wherein gag, pol and nef are expressed
		from three individual vectors.
35	86b, 88, 89	The claims are drawn to a multivalent vaccine wherein gag, pol and nef are expressed
		from one individual vectors.
36	86c, 88	The claims are drawn to a multivalent vaccine wherein gag, pol and nef are expressed
		from two individual vectors, one expressing nef-pol fusion and one expressing gag.
37	86d, 87, 88	The claims are drawn to a multivalent vaccine wherein gag, pol and nef are expressed
		from two individual vectors, one expressing gag-pol fusion and one expressing nef.
38	86e, 88	The claims are drawn to a multivalent vaccine wherein gag, pol and nef are expressed
		from two individual vectors, one expressing nef-gag fusion and one expressing pol.
39	86f, 88	The claims are drawn to a multivalent vaccine wherein gag, pol and nef are expressed
		from a single vectors as a fusion protein.
40	86g, 88	The claims are drawn to a multivalent vaccine wherein gag and pol are expressed
		from two individual vectors.
41	86h, 88, 89	The claims are drawn to a multivalent vaccine wherein gag and pol are expressed
		individually from one vector.
42	86i, 88	The claims are drawn to a multivalent vaccine wherein pol and nef are expressed
		from two individual vectors.
43	86j, 88, 89	The claims are drawn to a multivalent vaccine wherein pol and nef are expressed
		from individually from one vector.
44	86k, 88	The claims are drawn to a multivalent vaccine wherein nef and gag are expressed
		individually from one vector.
45	861, 88, 89	The claims are drawn to a multivalent vaccine wherein nef and gag are expressed
-	1,,	individually from one vector.
46	86m, 88	The claims are drawn to a multivalent vaccine wherein gag and pol are expressed as
	002,00	fusion protein from one vector,
47	86n, 88	The claims are drawn to a multivalent vaccine wherein pol and nef are expressed as
••	1 3324 30	fusion protein from one vector.
48	860, 88	The claims are drawn to a multivalent vaccine wherein <i>nef</i> and <i>gag</i> are expressed as a
	1 000, 00	fusion protein from one vector.
		1 motor protein from one recent.

The inventions listed as Groups 1-48 do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The technical feature linking groups 1-33 appears to be a recombinant adenoviral vector wherein the adenoviral vector is at least partially deleted in E1 but the vector may contain more deletions, the vector contains wild type sequences including packaging signals and a gene encoding a heterologous HIV protein or fragments thereof. Ertl et al. (WO 96/39178) disclose a recombinant adenoviral vector that is deleted in E1 and partially deleted in E3, the remainder of the adenoviral vector contains wild type sequences. The vector additionally contains an insertion of a heterologous protein which includes HIV proteins (see abstract and claims 1 and 5). Therefore, the technical feature linking the inventions of groups 1-45 does not constitute a special technical feature as defined by PCT Rule 13.2, as it does not define a contribution over the prior art.

The special technical feature of the following groups 1-3, 7-15, 19-30 and 34-48 is considered to be the combination of sequences that is disclosed in each group, see individual claim groupings above for the different sequences. The DNA disclosed in each group is made up of a different sequence having a different structure and different function.

International application No.

PCT/US01/28861

The special technical feature of group 4, 16 and 31 is considered to be a method of producing recombinant adenoviral particles. Each group contains different sequences hence the resulting particles would have different structures and functions associated with the particle.

The special technical feature of group 5, 17 and 32 is considered to be a method of producing a cellular mediated immune response to the heterologous protein encoded by the different adenoviral vectors. Each group contains different sequences a encoding different protein, therefore the resulting immune response will also be different.

The special technical feature of group 6, 18 and 33 is considered to be a method of producing a cellular mediated immune response to the heterologous protein encoded by the different ademoviral vectors in conjunction with immunizing the individual a DNA plasmid vaccine. Each method contains different sequences encoding a different protein, therefore the resulting immune response will also be different.

Accordingly, groups 1-48 are not so linked by the same or corresponding technical feature as to form a single general inventive concept.

Continuation of B. FIELDS SEARCHED Item 3:

WEST 2.0, STN-BIOSIS, MEDLINE

adenoviral vector, deletion, HIV, Gag, polyadenylation signal, CMV promoter